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Livestock foraging behavior in response to sequence and interactions among alkaloids, tannins, and saponins

Tiffany L. Jensen

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LIVESTOCK FORAGING BEHAVIOR IN RESPONSE TO SEQUENCE AND INTERACTIONS

AMONG ALKALOIDS, TANNINS, AND SAPONINS

by

Tiffanny L. Jensen

A dissertation submitted in partial fulfillment
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in

Range Science

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UTAH STATE UNIVERSITY
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ABSTRACT

Livestock Foraging Behavior in Response to Sequence and Interactions

Among Alkaloids, Tannins and Saponins

by

Tiffanny Lyman Jensen, Doctor of Philosophy

Utah State University, 2012

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The influence of primary compounds (energy, protein, minerals, and vitamins) in animal nutrition and foraging behavior has been studied for years. The roles of secondary compounds (terpenes, alkaloids, and phenolic compounds) are equally important, yet they have been ignored until recently. Where secondary compounds were once considered toxic by-products of plant metabolism, we now know they are actively involved in plant and animal behavior, health, and productivity. Though often appreciated historically for their negative impacts on food intake and animal production, we are becoming increasingly aware of their beneficial roles in the health of plants, animals, and humans. When animals can ingest an array of plants that contain different kinds and amounts of secondary compounds, they can mix different foods in their diet to better use both primary and secondary compounds, enhancing their health and production, as well as economic and ecological characteristics of landscapes.

Endophyte-infected tall fescue contains the alkaloids perlolidine, perloline, ergotamine, and ergovaline, which are all steroidal or protein-like in nature. Tannins and saponins have a high affinity for binding proteins and lipids in the gastro-intestinal tract of animals, and facilitating their excretion from the body. These findings suggest animals may increase their use of forages with alkaloids when they are also provided forages that contain tannins or saponins. The sequence in which forages with different secondary compounds are ingested may influence any potential interactions because different compounds have different residence times in the gastrointestinal tract.

I tested the hypothesis that cattle and sheep foraging behavior is influenced by eating different combinations of forages containing secondary compounds in different sequences. In pen and pasture trials, I showed that 1) cattle grazing pastures of endophyte-infected tall fescue (TF) grazed more often on TF when first allowed to graze legumes containing either tannins or saponins, and they grazed TF much more when they first grazed legumes as opposed to the reverse sequence; 2) sheep fed basal diets high in the alkaloid ergotamine d tartrate (EDT) ate more when supplemented with food containing either tannins or saponins, but in contrast to the trials on pasture with cattle, their behavior was not dramatically influenced by sequence; 3) cattle fed fresh cut endophyte-infected tall fescue were not influenced by the addition of tannin to their drinking water, as tannins limited both water and forage intake; 4) sheep fed food containing EDT ate more when supplemented with food containing tannins or when given a choice of foods containing tannins or saponins, than sheep supplemented with food containing saponins or no additional secondary compound. Results from these studies suggest that secondary compounds interact with one another to influence foraging behavior, and that sequence of food ingestion can be influential when animals graze on pastures.

(109 pages)

PUBLIC ABSTRACT

Livestock Foraging Behavior in Response to Sequence and Interactions

Among Alkaloids, Tannins, and Saponins

by

Tiffanny L. Jensen, Doctor of Philosophy

During the past several decades, people worldwide have expressed a growing interest in reconstructing ecosystems to enhance ecological, economic, and social values. Yet, to do so we must find ways to enhance biodiversity, environmental quality and the sustainability of grazing lands. In all these instances, plants are the glue that binds soils, water, herbivores, and people. However, monocultures or simple grass-legume mixtures are not always ideal for intensively managed pastures due to their seasonality, susceptibility to pests, and monotony of plant nutrients and toxins. All plants contain so-called “toxins,” more appropriately referred to as secondary compounds, which are crucial in plant defense and survivability. High doses of secondary compounds can cause decreases in animal intake, production, and health, dramatically impacting the efficiency and cost of livestock production and land management. Yet, diverse mixtures of plants may provide many benefits monocultures cannot. Complementarities among plant secondary compounds may actually enhance animal intake, efficiency and health when animals eat plants that contain higher levels of secondary compounds (i.e. toxins).

With my PhD program, I sought to understand how complementary interactions among the secondary compounds alkaloids, tannins, and saponins influenced livestock grazing behavior and the further implications of these interactions for land and animal management. This

research will help us better understand the ability of herbivores to use complementary forages to enhance the biodiversity of landscapes and productivity of herbivores while at the same time decreasing our reliance on herbicides and insecticides to protect plants. Complementary foods and sequence in animal grazing systems may prove fundamental in the upcoming transition from high fossil fuel inputs to more sustainable alternatives in animal and land management.

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Tiffanny L. Jensen

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CHAPTER 1

INTRODUCTION

Life sustains life through myriad interactions among soils, plants, animals, and people. These interactions impact all aspects of agriculture, natural resource management, and human health. Chemical and microbial interactions in soils influence the biological workings of plants which then influences the nutrition and behavior of animals, which further impacts the health and vitality of people. Reconstructing ecosystems to enhance ecological, economic, and social values relies on the understanding and appreciating the complex interactions involved in the biodiversity and sustainability of natural resources.

Most agricultural production in the Intermountain West revolves around livestock grazing pastures and rangelands. While commonly encouraged historically, planting monocultures or simple grass-legume mixtures is not always ideal for intensively managed pastures, because of their seasonality, susceptibility to pests, and monotony of their nutrient profiles. Diverse mixtures of plants may provide benefits monocultures cannot. For instance, more diverse mixtures of plants are likely to capture and use nitrates that accumulate in the root zone during the growing season, so they are not leached by winter precipitation (MacAdam, 2002). A diverse mix of species may add biochemical structure, nutrient diversification, and cover during times of the year when such resources may be absent if producers rely on monocultures. Furthermore, diverse mixtures of plants allow individual animals to select foods to meet their unique dietary needs, benefiting the physical health of the animal, the environmental health of landscapes, and the economic health of the producer.

Plants and herbivores interact one with another in complex and often subtle ways mediated by interactions among primary (PC) and secondary compounds (SC). Primary compounds (energy, protein, minerals and vitamins) directly influence the nutritional status of an animal. So-called SC (alkaloids, terpenes, and phenolics), formerly thought to be waste products of plant metabolism, are involved in plant defense, attracting pollinators, and a variety of other plant functions (Rosenthal and Berenbaum, 1992). Environmental factors, including herbivory and availability of nutrients, water, and light, influence the evolution (Coley et al., 1985) and phenotypic expression (Bryant et al., 1983) of SC, which then influence how SC interact with soils, plants, herbivores, and people (Provenza, 2008).

Historically, ecologists emphasized the role of SC in plant defense and herbivore behavior. Most SC limit how much of a particular plant species an herbivore can eat, thereby spreading the load of herbivory across many species and encouraging herbivores to eat a variety of plants (Freeland and Janzen, 1974, Foley et al., 1999). Post-ingestive feedback mechanisms assist animals in limiting the dose of primary or SC to avoid toxicosis (Provenza, 1995, 1996; Foley et al., 1999). For instance, oral gavages of toxins cause dose-dependent decreases in intake of foods that contain the toxins (Wang and Provenza, 1997). Yet, herbivores are able to eat more when given a variety of food with different kinds of SC as different SC affect the body in different ways and are detoxified by different mechanisms (Freeland and Janzen, 1974). A diverse intake of SC may also lead to neutralization or inactivation of the compound, which in turn could reduce susceptibility to toxic doses.

Interactions among plants with SC can lead to complementary relationships such that eating a combination of foods may exceed the benefit of consuming any one food in isolation (Tilman, 1982). When sheep choose between foods that contain either amygdaline or lithium chloride, they eat more than lambs offered a food that contains only one of these compounds; the same is true with nitrate and oxalate (Burritt and Provenza, 2000). Sheep eat more when offered three foods that contain terpenes, tannins, and oxalates than when offered foods with only one or two of these compounds (Villalba et al., 2004). They also eat more food containing alkaloids when supplemented with foods containing tannins or saponins than when only given a food with alkaloids (Lyman et al., 2008). Mule deer eat more when offered both sagebrush and juniper (12.3 g/kg BW), plants that contain different phenolics and terpenes, than when they are offered only sagebrush (4.2 g/kg BW) or juniper (7.8 g/kg BW) (Smith, 1959). Brushtail possums that can select from two diets containing phenolics and terpenes eat more food than when they consume diets containing only one of these compounds (Dearing and Cork, 1999), and the same is true in principle with squirrels (Schmidt et al., 1998).

The sequence in which complementary foods are ingested also influences preference. Sheep eat more terpene-containing foods after a meal of tannin-containing foods than when the sequence is reversed (Mote et al., 2008). Cattle graze more on endophyte-infected tall fescue (containing alkaloids) when first allowed to graze on legumes containing tannins or saponins than when the sequence was reversed (Lyman et al., 2011), and sheep decrease intake of tall fescue in a meal, unless they receive an oral gavage of tannins prior to the meal, in which case they eat tall fescue throughout the meal (Lisonbee, 2009). Thus, while animals can meet needs for PC and tolerate higher total intake of SC when they can choose from a variety of plants (Provenza and Villalba, 2006), outside of the aforementioned studies, very little is known about which SC in plants are complementary and which are not when different forages are ingested in different sequences.

In their efforts to describe the defensive roles of SC in plants, researchers have not considered their possible health benefits (Engel, 2002). Everything depends on concentration: PCs and SCs at too high concentrations can be toxic, while at lower concentrations they can both have health benefits (Provenza and Villalba, 2006). Likewise, in our haste to increase the palatability of monoculture pasture species, researchers and producers have selected for low concentrations of compounds such as alkaloids (reed canary grass and endophyte-infected tall fescue), tannins (birdsfoot trefoil), and saponins (alfalfa), not appreciating that these compounds in proper mixtures may actually benefit both plants and animals.

My objectives were to test the hypothesis that cattle and sheep foraging behavior is influenced by eating different combinations and sequences of forages containing SC. Specifically I determined: 1) if cattle ate more endophyte-infected tall fescue after first grazing on legumes containing either tannins (birdsfoot trefoil) or saponins (alfalfa) than cattle grazing in the reverse sequence; 2) if sheep fed a food containing an alkaloid (ergotamine d tartrate) ate more when first supplemented with food containing either tannins or saponins than when fed in the reverse sequence; 3) if cattle ate more freshly cut endophyte-infected tall fescue when supplemented with tannin added to their water than cattle only given fresh water; 4) and if sheep varied their intake of foods containing ergotamine d tartrate, tannins, and saponins when given in various combinations and sequences.

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CHAPTER 2

CATTLE PREFERENCES DIFFER WHEN ENDOPHYTE-INFECTED TALL FESCUE, BIRDSFOOT TREFOIL, AND ALFALFA ARE GRAZED IN DIFFERENCE SEQUENCES[1]

Abstract

We determined if sequence of ingestion affected use of endophyte-infected tall fescue (TF) when cattle also grazed either birdsfoot trefoil (BFT) or alfalfa (ALF). Based on chemical characteristics of TF (alkaloids), BFT (tannins), and ALF (saponins), we hypothesized that cattle first allowed to graze ALF or BFT would subsequently spend more time grazing TF than cattle that first grazed TF followed by ALF or BFT. Sixteen bred heifers (478± 39 kg initial BW) were randomly assigned to 4 replicated pasture units. Each replicated unit consisted of 4 treatment sequences (TF → BFT, TF→ALF, BFT→TF, or ALF → TF), with 2 cows per sequence. Pastures were in the vegetative stage of growth at a height of 20 to 30 cm and provided ad libitum forage to cattle. We recorded foraging on TF, BFT and ALF using scan sampling of individuals at 2-min intervals. The study was conducted in 4 phases run sequentially, for a total of 30 d. In phases 1 and 3, cattle in Group 1 grazed TF pastures for 45 min and were then moved to BFT pastures for the next 45 min (TF→BFT); cattle in Group 2 grazed in the reverse sequence (BFT→TF). Inphases 2 and 4, cattle in Group 1 grazed TF pastures for 45 min and then subsequently grazed ALF pastures for the remaining 45 min (TF→ALF); cattle in Group 2 grazed in the reverse

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[1] Chapter 2 Coauthored by Tiffanny Lyman Jensen, Frederick D. Provenza, and Juan J. Villalba. J. Anim. Sci. published online Nov. 5, 2010.

sequence (ALF→TF). Sequence of plant ingestion affected food selection. In phase 1, scans revealed grazing of TF by heifers was cyclic, and heifers tended to have more scans grazing TF when they grazed BFT→TF; scans for heifers grazing TF were consistently ALF→TF spent considerably more scans foraging on TF from d 4-10 than heifers that grazed in the sequence TF→ALF, and they remained higher throughout Phase 4 of the trial. While the sequence ALF→TF appeared to be more effective than BFT→TF, consistent with the hypothesis of a complementary relationship between the steroidal alkaloids in TF and saponins in ALF, tannin concentrations in BFT were low (1.8%), which likely reduced the presumed inactivation of alkaloids by tannins. We also speculate heifers needed to learn about the positive post-ingestive influence of sequence, a notion consistent with more similar scans spent foraging BFT and TF early in Phases 1 (BFT→TF) and 2 (ALF→TF), and with the consistent and marked increase in scans spent foraging on TF for animals foraging in Phases 3 (BFT→TF) and 4 (ALF→TF).

INTRODUCTION

Little is known about how the sequence of food ingestion influences forage intake and preference, though it appear to be important. Sheep eat more when foods with secondary compounds are offered in the morning followed by limited nutritious foods in the afternoon (Papachristou et al., 2007). Sheep also eat more food with terpenes when they first eat food with tannins (Mote et al., 2008); food with tannins eaten first remains in the gut up to 72 hours (Silanikove et al., 1994), where tannins can interact with terpenes, whereas terpenes are highly soluble compounds absorbed quickly from the gastrointestinal tract and eliminated from the body (Foley and McArthur, 1994). Altering the sequence of forage ingestion also causes calves to spend more time eating grasses such as endophyte-infected tall fescue and reed canarygrass when they first eat legumes such as alfalfa or birdsfoot trefoil and then eat endophyte-infected tall fescue or reed canarygrass (Lyman, 2008).

Our objective in the present study was to follow-up on the study of Lyman (2008), which was more elaborate in the choices offered to fall-born calves, with a specific focus on foraging sequence and more limited choices offered to bred heifers. To do so, we determined if the sequence of grazing endophyte-infected tall fescue (TF) and legumes containing tannins (birdsfoot trefoil, BFT) or saponins (alfalfa, ALF) increased use of TF by bred heifers. The alkaloids in TF are protein-like and steroidal in nature, whereas the tannins in BFT and the saponins in ALF are high molecular weight compounds, not absorbed through the rumen wall, with high affinity for binding to protein and lipid-like compounds such as the alkaloids in TF (Malinow et al., 1979; Jones and Mangan, 1977). We thus hypothesized cattle would eat more TF after eating forages with tannins (BFT) or saponins (ALF).

MATERIALS AND METHODS

Chemical Characteristics of Plant Species. Alkaloids are small, fat-soluble molecules absorbed rapidly through the rumen epithelium. In high amounts, they can decrease food intake and animal performance (Cheeke and Schull, 1985). Tall fescue contains two groups of alkaloids, one associated with the plant and the other allied with the fungus *Neotyphodium coenophialum*. The intrinsic alkaloids perlolidine and perloline, which are steroidal, negatively affect rumen fermentation and food intake. The fungus-associated alkaloids N-acetylloline, N-formylloline, ergotamine and ergovaline, which have lipid structures, reduce food intake and cause fescue toxicity.

Conversely, tannins and saponins are high-molecular-weight compounds (2,000 to 4,000) that remain in the gut for many hours where they interact with many other compounds (Kumar and Singh, 1984; Min and Hart, 2003). Condensed tannins in plants like BFT bind to proteins in the rumen (Jones and Morgan, 1977); as alkaloids are nitrogen-based, we hypothesized including tannin-containing BFT in the diets of livestock would neutralize the alkaloids in TF, and stable complexes form between alkaloids and tannins (Okuda et al., 1982; Wong and Provenza, unpublished data). Saponins in plants like alfalfa bind to lipids such as cholesterol in the gastro-intestinal tract of animals causing their excretion in the feces (Malinow et al., 1979); as the endogenous alkaloids in TF are lipid, we hypothesized including ALF in the diet of animals grazing TF would also neutralize the alkaloids in TF. Given the rapid rate of absorption of alkaloids relative to tannins and saponins, we hypothesized that first grazing BFT or ALF would increase concentrations of tannins and saponins in the rumen and enable heifers to better use TF.

Pasture Design. Plant species with alkaloids, tannins, and saponins were seeded at the Utah Agricultural Experiment Station Pasture Research Facility in Lewiston, UT (41’57 N. 111’52 W.). In 2006, we planted monocultures of tall fescue (TF) (*Festuca arundinaceae, Kentucky 31 endophyte-infected*) (Rottinghaus et al., 1991) with high concentrations of alkaloids, birdsfoot trefoil (BFT) (*Lotus corniculatus variety Goldie*) with high tannins (Hedqvist et al., 2000; Terrill et al., 1992), and alfalfa (ALF) (*Medicago sativa varieties Vernal and Lahontan*) with high saponins (Pedersen, 1975; Pedersen et al., 1976).

Pasture units, constructed with temporary electric fence, consisted of a 0.05 ha plot of TF, a 0.04 ha plot of ALF and a 0.04 ha plot of BFT planted in adjacent monoculture strips, with 2.25 ha of orchard grass (OG) as a holding area (Figure 1). We had 4 replications of each unit with the following sequences of grazing: 1) TF followed by (→) ALF or BFT (Sequence 1), and 2) ALF or BFT followed by (→) TF (Sequence 2).

Grazing Trials. Sixteen bred 2-yr-old Black Angus heifers (478± 39 kg initial BW) were used in all phases of the trials. We randomly assigned 4 heifers to each of the 8 pasture units, with 2 heifers in each sequence within a unit. Each morning, cattle were moved from adjacent OG pastures to trial pastures for their morning meals. After each morning trial, cattle were moved back into OG pastures, where water and mineral supplements were provided. Pastures were in the vegetative stage of growth at a height of 20 to 30 cm and all plots provided ad libitum forage to cattle. Cattle were weighed pre- and post-trial to estimate changes in body weight during the 30-d trials. Procedures followed the protocols for animal care and use (IACUC protocol approval number 1372).

<u>Phase 1:</u> Tall Fescue and Birdsfoot Trefoil. In the first phase, which lasted 10 d, cattle in Group 1 grazed TF pastures for 45 min and were then moved to BFT pastures for the next 45 min (Sequence 1 TF→BFT). Cattle in Group 2 grazed in the reverse sequence (Sequence 2 BFT→TF).

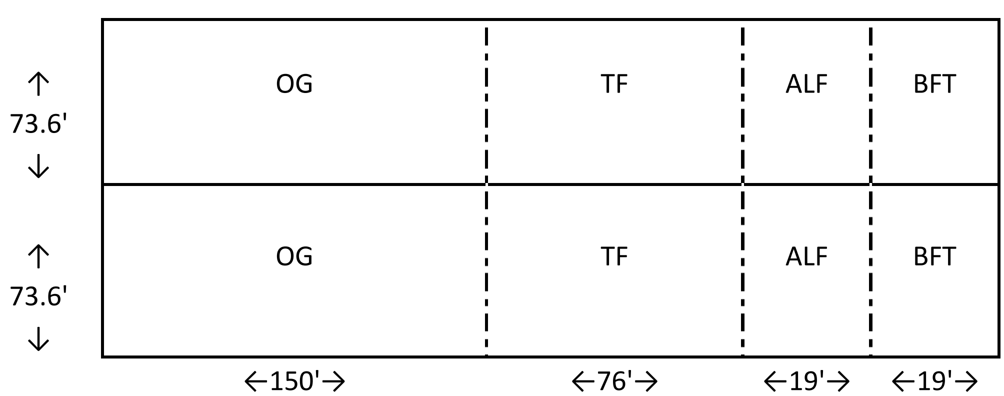

Figure 1. Depiction of one pasture unit containing adjacent monoculture pastures of Tall Fescue (TF), Alfalfa (ALF), and Birdsfoot Trefoil (BFT) with Orchard Grass (OG) holding areas. Within each pasture unit were 2 sequence treatments (Seq 1: TF → BFT or ALF and Seq 2: BFT or ALF → TF).

Phase 2: Tall Fescue and Alfalfa. In the second phase, which also lasted 10 d, cattle in Group 1 grazed TF pastures for 45 min and then subsequently grazed ALF pastures for the remaining 45 min (Sequence 1 TF→ALF). Cattle in Group 2 grazed in the reverse sequence (Sequence 2 ALF→TF).

Phase 3: Tall Fescue and Birdsfoot Trefoil (5 d). Given the experience animals gained in Phase 1, we wanted to determine if their behavior was similar in Phase 3 to that exhibited in Phase 1. The third phase thus consisted of one 5-d period where cattle in Group 1 first grazed TF pastures for 45 min and then grazed BFT for 45 min for a 5 d period (Sequence 1 TF→BFT). Cattle in Group 2 grazed in the reverse sequence (Sequence 2 BFT→TF).

Phase 4: Tall Fescue and Alfalfa (5 d). Given the experience animals gained in Phase 2, we wanted to determine if their behavior was similar in Phase 4 to that exhibited in Phase 2. The fourth phase thus consisted of another 5-d period where cattle in Group 1 first grazed TF pastures for 45 min and then grazed ALF for 45 min for a 5 d period (Sequence 1 TF→ALF). Cattle in Group 2 grazed in the reverse sequence (Sequence 2 ALF→TF).

Scan Samples. In all phases of the study, one observer recorded behavioral data using scan-samples of individually marked animals at 2-min intervals throughout daily trials from 0600 to 1030 each day (Altman, 1974). Scans were taken from a 3 m high platform centrally located to enable the observed to see all of the animals and whether or not they were grazing a particular forage. Animals were considered to be grazing only when they were actually biting and chewing forage; no grazing scan was recorded if an animal had its head in the sward, but was not biting or chewing forage. We then calculated the percent of scans each animal spent grazing each forage each day.

Chemical Composition of the Forages. Representative forage samples, collected from plants along a paced transect across each pasture, were hand-harvested at the end of the study, placed in plastic bags covered with dry ice immediately after harvest, and transported to a freezer where they were kept at -20 ^{o}C. They were subsequently freeze dried, ground through a Wiley mill with a 1-mm screen, and analyzed for neutral detergent fiber (NDF; Goering and Van Soest, 1970) and nitrogen (Method 990.03, AOAC, 2002), as well as condensed tannins (BFT) (Terrill et al., 1992), saponins (ALF) (Lee et al., 2001) and the alkaloid ergovaline (TF) (Rottinghaus et al., 1991).

Statistical Design. The statistical design for the analysis of variance was a repeated measures with 4 replications of 2 sequences (TF→ legume or legume → TF). Day was the repeated measure. Separate analyses were carried out for phases 1, 2, 3, and 4. The response variable was percent of scans observed per forage.

RESULTS

Chemical Composition of the Forages. Tall fescue contained more fiber and less N than either ALF or BFT (Table 1). Tall fescue was high in ergovaline, and ALF contained high amounts of saponins, but BFT was low in tannins (Table 1).

Phase 1. Percent scans grazing TF did not differ between groups when cattle grazed TF→BFT or BFT→TF. Heifers spent 36% of the scans grazing TF in the sequence TF→BFT and 45% of the scans grazing TF in the sequence BFT→TF (*P = 0.52*, Figure 2A). Nor did scans spent grazing BFT differ (TF→BFT= 98% vs. BFT→TF = 96%, *P = 0.27*). Neither day (*P = 0.36*) nor day x sequence differed (P = 0.27).

Phase 2. The percent scans grazing TF differed between groups when heifers grazed TF→ALF as opposed to ALF→TF. Heifers spent 28% of the scans grazing TF in the sequence TF→ALF and 51% of the scans grazing TF in the sequence ALF→TF (*P =0.03*, Figure 2C). Groups did not differ in scans grazing ALF (TF→ALF= 98% vs. ALF→TF= 98%, *P = 0.52*). Day (*P <0.0001*) and day x sequence (*P = 0.03*) differed.

Phase 3. When heifers grazed TF and BFT a second time, the percent of scans grazing TF tended to differ between groups for TF→BFT and BFT→TF. Heifers grazed 37% of the scans on TF in the sequence TF→BFT and 61% of the scans on TF in the sequence BFT→TF (*P = 0.18*, Figure 2B). Groups did not differ in scans grazing BFT (BFT→TF =98%, TF→BFT = 95%, *P= 0.27*). Day (*P<0.05*) differed, but no day x sequence interaction was observed (P = 0.27).

Table 1: Plant chemical analyses (means ± std. errors) for neutral detergent fiber (NDF), nitrogen (N), and for alkaloids (endophyte-infected tall fescue, *Festuca arundinaceae, Kentucky 31)*, tannins (birdsfoot trefoil, *Lotus corniculatus variety Goldie*) and saponins (alfalfa, *Medicago sativa variety Vernal*).

Plant Species	NDF, %	N, %	Total Condensed Tannins, %	Saponins, %	Ergovaline, ppb
Birdsfoot Trefoil	44.1±2.5	3.4±0.6	1.81±0.5	--	--
Alfalfa	42.2±5.2	3.8±0.5	--	3.9± 0.6	--
Tall Fescue	59.8±3.0	1.9±0.4	--	--	263±52.7

Phase 4. When heifers grazed TF and ALF a second time, the percent of scans grazing TF differed between groups for TF→ALF and ALF→TF. Heifers grazed 55% of the scans on TF in the sequence TF→ALF and 85% of the scans on TF in the sequence ALF→TF (*P = 0.05*, Figure 2D). Groups did not differ in scans grazing ALF (ALF→TF =98%, TF→ALF = 98%, *P= 0.52*). Day differed (*P<0.01*), but there was no day x sequence interaction (P = 0.67).

Weight gains. Cattle gained an average of 0.95 ± 0.54 kg/head/d.

DISCUSSION

We determined if foraging sequence and plant diversity enhanced use of TF when cattle foraged on legumes that contained tannins (BFT) or saponins (ALF), and we found that forage species and sequence of forage ingestion affected food selection. Scans spent grazing TF were cyclic, especially when heifers grazed TF→BFT in Phase 1, and they tended to spend more scans grazing TF when they grazed BFT→TF, particularly on d 3, 5, 7, 9 and 10 of Phase 1 (Figure 2A); importantly, scans spent grazing BFT were consistently higher throughout Phase 3 of the trial (Figure 2B), which is consistent with previous findings when calves grazed significantly more in the sequence BFT→TF than TF→BFT (Lyman, 2008). In Phase 2, heifers that grazed in the sequence ALF→TF spent considerably more scans foraging on TF from d 4-10 compared with heifers that grazed in the sequence TF→ALF, and they remained higher throughout Phase 4 of the trial (Figures 2C and 2D).

Scans spent grazing TF increased throughout the trials for animals grazing BFT→TF and ALF→TF, though heifers consistently grazed less on TF plots in phase 1 (BFT→TF) than in phase 2 (ALF→TF) (Figure 2A and 2C). Thus, while the sequence ALF→TF appeared to be more effective than BFT→TF, consistent with the hypothesis of a complementary relationship between the steroidal alkaloids in TF and saponins in ALF, tannin concentrations in BFT were low (1.8%), which may have reduced the presumed tannin-alkaloid interactions. Tannin concentrations of 6-10% appear to be most effective at influencing grazing behavior without causing any harmful effects on intake or performance (Jones and Mangan, 1977; Lyman et al., 2008). We also speculate heifers needed to learn about the positive post-ingestive influence of sequence for BFT and ALF on TF, a notion consistent with more similar scans spent foraging early in Phases 1 (BFT→TF) and 2 (ALF→TF), and with the consistent and marked increase in scans spent foraging on TF for animals foraging in the Phases 3 (BFT→TF) and 4 (ALF→TF).

Our findings thus support hypotheses that complementarities and foraging sequences influence scans spent feeding and forage intake, and they are consistent with findings that intake of high-alkaloid varieties of TF and reed canarygrass both increase when sheep eat legumes high in tannins (BFT) or saponins (ALF) (Owens, 2008). While these studies provide indirect evidence of secondary compound interactions for cattle or sheep, direct evidence comes from studies where lambs given intraruminal infusions of tannins or saponins increase use of endophyte-infected TF relative to lambs not infused with tannins or saponins when offered choices of BFT, ALF, TF and OG (Lisonbee et al., 2009; Villalba et al., 2010). They also come from studies where sheep eat more when offered foods high in tannins or saponins along with foods high in alkaloids (Lyman et al., 2008). This could occur because stable complexes form between alkaloids and tannins (Okuda et al., 1982; Wong and Provenza, unpublished data), and because alkaloids bind to saponins in the gastro-intestinal tract causing their excretion in the feces (Malinow et al., 1979).

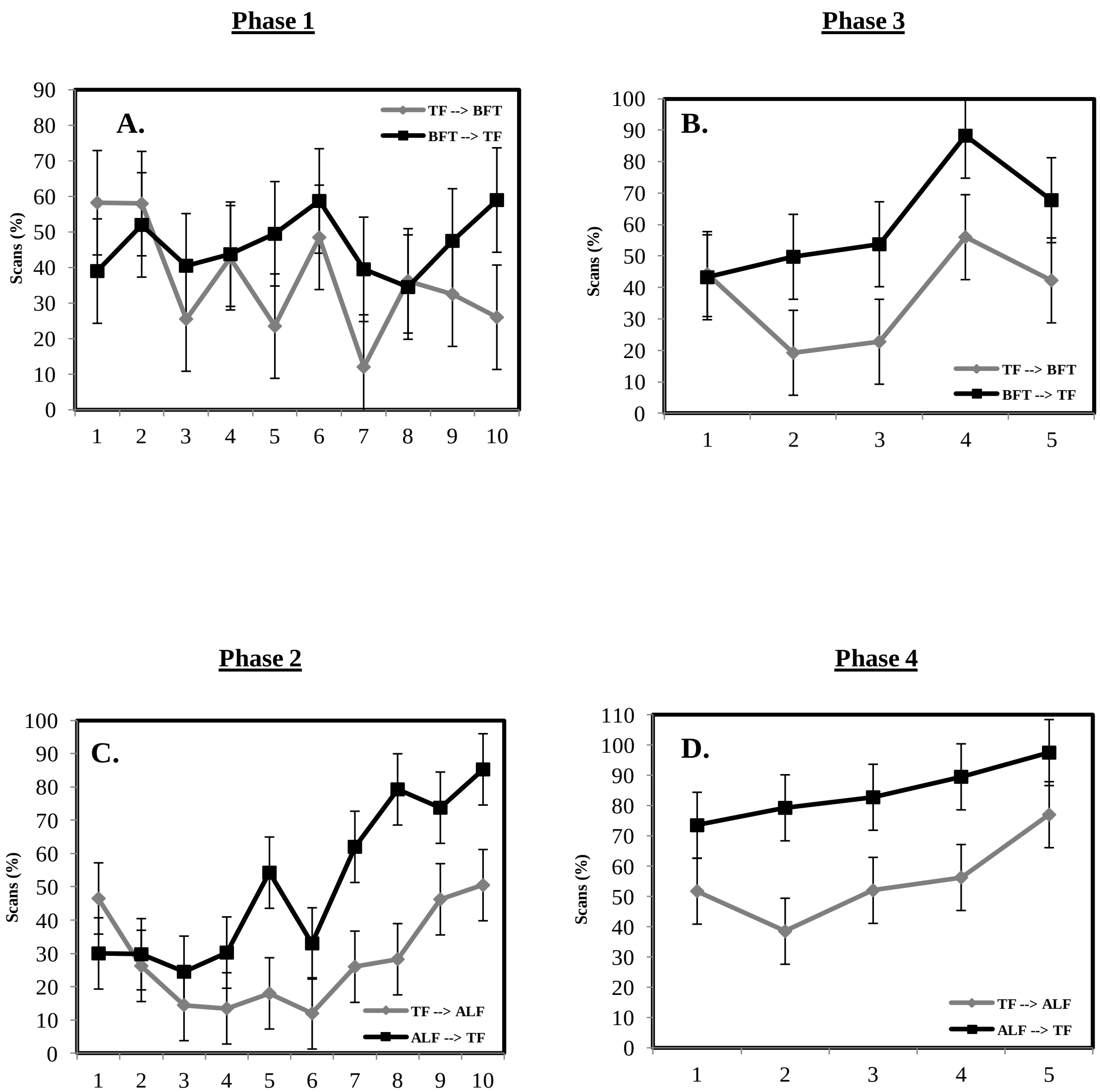

Figure 2. Percent scans heifers spent grazed tall fescue (TF) before or after a meal of birdsfoot trefoil (BFT) (A and B), or alfalfa (ALF) (C and D) in 4 phases. Phases 1 and 3 lasted 10 d, whereas Phases 2 and 4 lasted 5 d. Cattle first grazed TF for 45 min followed by a meal of BFT (A and B) or ALF (C and D) for 45 min or they first grazed BFT (A and B) or ALF (C and D) for 45 min followed by TF for 45 min. Bars are standard errors.

Obviously, more experimental analyses are necessary to assess the specific physiological and behavioral effects of interactions among secondary compounds, and to better understand higher-order interactions among primary and secondary compounds in various forages (Provenza et al., 2003). Both primary and secondary compounds in too great amounts can be toxic, whereas in appropriate amounts they interact to collectively benefit both nutrition and health (Provenza and Villalba, 2006, 2010). At the most simple levels, supplemental energy and protein enhance the abilities of animals to ingest forages high in secondary compounds, particularly when animals must eat a diet high in secondary compounds (e.g., Villalba et al., 2002ab; see review by Provenza et al., 2003). In our study, additional protein in the legumes likely contributed to the differences in use of TF as additional protein helps facilitate detoxification processes (Foley et al., 1994, 1999; Illius and Jessop, 1995), and protein supplementation increases intake of fiber (Van Soest, 1994). Meal size and length is larger in dairy cows fed a supplement before eating roughage than when the roughage is fed before the supplement (Morita et al., 1996). Whatever the higher-order interactions, findings from this and past studies show that forage complementarities and sequences facilitate intake of TF undoubtedly due to complex interactions among primary and secondary compounds.

IMPLICATIONS

Tall fescue is the primary grass growing on more than 14 million ha of pasture- and hay-land in the United States (Buckner et al., 1979). Most tall fescue is endophyte-infected, and the negative impact of tall fescue alkaloids on beef production was estimated at $600 million annually over 10 years ago (Paterson et al., 1995). A conservative estimate places the total livestock-related losses at $500 million to $1 billion a year (Univ. Nebraska, Institute of

Agriculture and Natural Resources). However, the alkaloids in TF, so problematic for ruminants, make the plant highly resistant to drought and other stresses. Improved seedling performance and survival, as well as insect and nematode resistance, drought resistance, improved nitrogen assimilation, higher seed set, and overall increased survival are all benefits from using endophyte-infected tall fescue in pasture systems (Pedersen et al., 1990).

Results from our study suggest ways for developing pastures and grazing systems that incorporate a variety of plant species with different secondary compounds to increase plant survivability as well as livestock productivity when dealing with forages such as TF. If, as our results suggest, tannin- or saponin-containing legumes can partially offset the negative effects of the alkaloids in TF, then new avenues for coping with fescue toxicosis would become available to producers willing to plant mixtures of forages. Our findings thus have the potential to create positive impacts on the economic and environmental aspects of producing livestock on tall fescue pastures.

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CHAPTER 3

INFLUENCE OF DIET SEQUENCE ON INTAKE OF FOODS CONTAINING ERGOTAMINE D TARTRATE, TANNINS, AND SAPONINS BY SHEEP

Abstract

Plant secondary compounds (SC) play crucial roles in plant survival and defense against herbivores and in appropriate amounts SC provide health and nutritional benefits to herbivores. Thus, the presence of SC in grazing systems is important, provided means are found to enhance the benefits of SC while minimizing their negative impacts on herbivores. One approach to this challenge is to offer herbivores mixtures of forages with diverse and complementary SC and in the appropriate temporal sequence such that a combination of SC becomes less toxic than each of its components in isolation. Our objective was to determine if a diversity of SC (tannins, saponins, and alkaloids) ingested in different temporal sequences influenced food intake by sheep. In Experiment 1, three groups of lambs (n = 8/group) received a food containing the alkaloid ergotamine d tartrate (EDT) and the same food containing condensed tannins (TAN) in three different sequences. Group 1 received TAN for 30 min followed by EDT for 1 h (TAN→ EDT), Group 2 received EDT for 1 h followed by TAN for 30 min (EDT→TAN), and Group 3 (Control Group) received only EDT for the same periods of time (EDT→EDT). Experiment 2 was similar to Experiment 1 except a new group of lambs was used and saponins (SAP) replaced tannins. Sheep had higher intakes of alkaloid-containing food (EDT) when supplemented with TAN ($P = 0.0002$) or SAP ($P = 0.0979$) than when fed only EDT, regardless of the sequence in which the foods were presented. Sheep also had higher total intakes when supplemented with TAN than SAP. Ingesting TAN or SAP prior to ingesting EDT did not increase consumption of *EDT* compared with the reverse sequences; the long residence time of tannins and saponins in the

gastrointestinal tract may have attenuated differences in food intake for sequences TAN→EDT and SAP→EDT compared with EDT→TAN and EDT→SAP. The intake of *TAN* was higher for the sequence TAN→EDT than for EDT→ TAN (P=0.0336). Likewise, the intake of *SAP* was higher for the sequence SAP →EDT than for EDT→SAP (P = 0.0162). In summary, our study suggests eating food with tannins or saponins increases intake of food with alkaloids, and total food intake is higher when the diet contained tannins as opposed to saponins. Sequence increased intake of tannin- and saponin-containing foods, but not alkaloid-containing food.

Introduction

Plant secondary compounds (SC) limit how much of a particular plant an herbivore can eat, causing animals to regulate their intake of foods with SC and thus spread the load of herbivory across many plant species with different SC in a plant community (Freeland and Janzen, 1974; Foley et al., 1999). This is because diverse SC can be detoxified by different mechanisms, and they can be inactivated by chemical interactions, leading to greater intakes than when a single SC-containing food is ingested (Freeland and Janzen, 1974; Foley et al., 1999).

Interactions and complementarities among SC influence foraging behavior (Provenza et al., 2003). Rats eat more of a combination of foods containing tannins and saponins because tannins and saponins chelate in the gastrointestinal tract, reducing the negative effects of both components (Freeland et al., 1985). When lambs can choose between foods that contain either amygdalin or lithium chloride, they eat more than lambs offered a food that contains only one of these compounds; the same is true with nitrate and oxalate (Burritt and Provenza, 2000).

Sheep also eat more when offered three foods that contain terpenes, tannins, and oxalates than when offered foods with only one or two of these SC (Villalba et al., 2004), and they eat more endophyte-infected tall fescue then they receive intraruminal infusions of tannins or saponins (Villalba et al., 2011).

Complementarities among SC can potentially enhance food intake and reduce the negative impacts of SC on an herbivore’s body. However, little is known about interactions among SC and even less is known about how the sequence of eating foods with different SC affects foraging, both critical factors in considering the suitability of pastures and rangelands for the nutrition and health of herbivores (Provenza et al., 2007). Sheep eat much more food with terpenes when they first eat food with tannins (Mote et al., 2008). Cattle graze much more on forages containing alkaloids (reed canarygrass and tall fescue) when they are first allowed to graze legumes containing tannins (birdsfoot trefoil) and saponins (alfalfa) (Lyman et al., 2011b). Likewise, sheep supplemented first with tannins or saponins increase ingestion of alkaloid-containing food (Owens et al., 2012ab).

Landscapes that allow animals to select among alternative forages can thus enable individuals to meet needs for nutrients and to better cope with the negative effects of SC (Provenza et al., 2003, 2007). Shepherds in France strategically move their flocks within a sequential grazing rotation to stimulate the appetites of livestock, thus increasing nutrition and production, and to best use of all plant species in a community (Meuret, 2010). Thus, ingesting a variety of SC can reduce susceptibility to toxicosis (Provenza, 1996), and the sequence in which SC are ingested may also have an impact on animal and plant responses.

We showed previously that supplementing sheep with tannins or saponins increases intake of food containing alkaloids (Lyman et al., 2008), but we do not know if the sequence of food ingestion affects responses to alkaloids, tannins, and saponins. Based on biochemical complementarities among these compounds, we hypothesized that the sequence in which alkaloids, tannins, and saponins are ingested would influence the ability of sheep to consume these SC (Lyman et al., 2008).

Materials and Methods

Sheep were 6-month-old, commercial Dorper- Suffolk- Columbia crossbred lambs, averaging 35 kg (± 4.68 kg) during the first experiment and 32 kg (± 5.43 kg) during the second experiment. They were individually penned at the Green Canyon Ecology Center, Utah State University, Logan, Utah, U.S.A. with free access to trace mineralized salt blocks and fresh water. They were kept outdoors, under a protective roof in individual, adjacent pens measuring 2.4 x 3.6 m. All procedures were approved by the Utah State University Institutional Animal Care and Use Committee (IACUC #1417).

Experimental Design.

In two different experiments, run in 2 consecutive years, three groups of lambs (n = 8/group) received the same basal food -- containing either the alkaloid ergotamine d tartrate (EDT) or tannins (TAN) or saponins (SAP) -- in different sequences. Each experiment lasted 9 d, and a different group of lambs was used for each study. Lambs were offered 1000g of EDT for 1 h and 500g of TAN (Experiment 1), SAP (Experiment 2), or more EDT (Control Group) food for 30 min in two different sequences.

In Experiment 1, we fed sheep EDT supplemented with TAN in three different sequences. Group 1 received TAN for 30 min followed by EDT for 1 h (TAN→ EDT). Group 2 received EDT for 1 h followed by TAN for 30 min (EDT→TAN). Group 3 (Control Group) received

EDT for 30 min, followed by more EDT for the remaining hour of the trial (EDT→EDT). Experiment 2 was run the same as Experiment 1, but SAP replaced TAN as the supplement.

Foods.

Dried, ground alfalfa pellets and dried ground beet pulp were the ingredients of each food. For each experiment, a basal diet of 58% ground alfalfa and 38% ground beet pulp was mixed with ergotamine D tartrate (EDT) (Sigma-Aldrich Inc., St Louis, MO) at 30ppm, and 2% onion powder to act as a flavor cue that signaled the addition of EDT to the food. The tannin food (TAN) was 54% ground alfalfa, 34% ground beet pulp and 10% tannins. The saponin food (SAP) contained 58% ground alfalfa, 38% ground beet pulp and 2% saponin. All diets were mixed with 2% vegetable oil to reduce dust and inhalation of SC, particularly saponins, which tended to form a fine dust when mixed with food.

The condensed tannin was extracted from *Aspidosperma quebracho* (Tannin Corporation, Peabody, MA). Quebracho tannin is a complex of tannins, flavonoids, and other phenolic compounds (Mole and Waterman, 1987) containing approximately 85% condensed tannin (Titus and Provenza, unpublished results). The saponin was extracted from *Quillaja saponaria* (Sigma-Aldrich Inc., St Louis, MO). Tannins, saponins, and ergotamine D tartrate were all fine powders, which we thoroughly mixed with the other food ingredients that were ground to 1-2 mm particle size.

The concentrations of SC we used correspond with levels in pasture species with known effects on herbivores. Ergotamine at 30 ppm causes physiological changes similar to those observed in animals suffering from fescue toxicosis (Osborn et al., 1992). Tannin concentrations of 10% are in the range of Lotus spp. (Min and Hart, 2003), and saponins at 2% decrease food intake by sheep (Burritt and Provenza, 2000).

Diets containing alkaloids, tannins, and saponins were fed to sheep *ad libitum*. Food refusals were weighed to determine if lambs ate more of EDT food when first given tannin or saponin food than when given EDT food second in the sequence.

Statistical Analyses.

Data was analyzed using a mixed-effects model that accounted for the random effect of lamb (experimental unit) within sequence, and the fixed effects of sequence, day, and their interaction. The response variables were the amount (in Kg/BW) of food consumed (containing EDT, tannin, or saponin and the total amount of food consumed/day). Analyses were run with the General Linear Model procedure (GLM) of SAS (SAS Inst., Inc. Cary, NC; Version 9.1 for Windows). Model diagnostics included testing for a normal distribution of the error residuals and homogeneity of variance. No transformation was required to meet these assumptions. When the main effect was significant ($P < 0.05$), means were compared using least significant differences.

Results

Experiment 1: EDT and Tannin

Ergotamine Intakes. Sheep supplemented with TAN ate more EDT than sheep fed only EDT (P = 0.0002). Sheep fed TAN→EDT ate 17 g EDT/kg BW/d, sheep fed EDT→ TAN ate 18.5 g EDT/kg BW/d, and sheep fed EDT→EDT ate only 8.6 g EDT/kg BW/d. Intake of EDT varied across days (P<0.0001), and Group interacted with Day (P=0.0001; Figure 3).

Tannin Intakes. Averaged across days, intake of TAN tended to be higher for TAN→EDT (7 g TAN/kg BW/d) than for EDT→ TAN (5 g TAN/kg BW/d) (P = 0.2190). However, intake of TAN varied across days (P=0.0032), and Group interacted with Day such that intake of TAN→EDT was higher than intake of EDT→ TAN on most days (P=0.0336; Figure 4).

Total Food Intakes. Sheep fed TAN and EDT ate more total food than sheep fed only EDT (P=0.0223). On average, TAN→EDT ate 24g of food/kg BW/d, EDT→TAN ate 23 g food/kg BW/d, and EDT→EDT ate only 17 g food/kg BW/d. Intake varied across days (P<0.0001), and Group interacted with Day (P<0.0001).

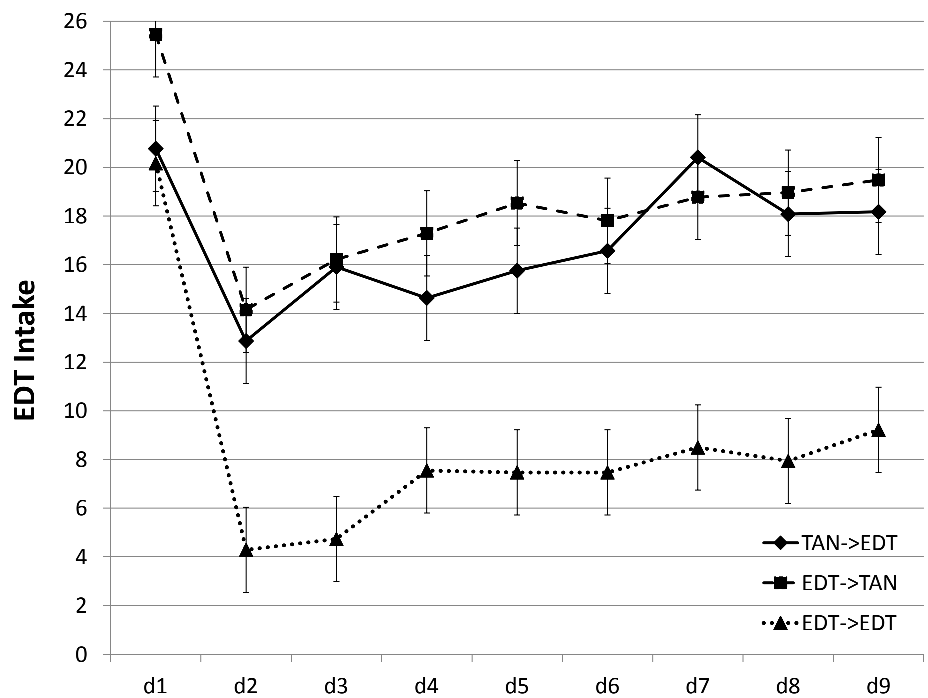

Figure 3. Intake of food containing ergotamine d tartrate (EDT) by sheep during Experiment 1. Sheep were supplemented with food containing tannins before EDT food (TAN→ EDT), with tannin after EDT (EDT→ TAN), or with only EDT (EDT→EDT). Bars are standard errors.

Experiment 2: EDT and Saponin

Ergotamine Intakes. Sheep fed SAP ate more EDT than sheep fed only EDT (P = 0.0979). Sheep fed SAP→EDT ate 10 g EDT/kg BW/d, sheep fed EDT→SAP ate 12 g EDT/kg BW/d, and sheep fed EDT→EDT ate only 7 g EDT/kg BW/d. Intake of EDT varied by day (P<0.0001) and Group and Day did not interact (P=0.5957; Figure 5).

Saponin Intakes. Sheep fed SAP→EDT ate more SAP than sheep fed EDT→SAP (P = 0.0162; 6 vs. 2 g SAP/kg BW/d). Intake of SAP varied across days (P<0.0001), and Group and Day tended to interact (P=0.0978; Figure 6).

Total Food Intakes. Total food intake did not differ among groups (P=0.7329). On average, sheep fed SAP→ EDT ate 14g of food/kg BW/d, sheep fed EDT→SAP ate 17 g food/kg BW/d, and sheep fed EDT→EDT ate 16g food/kg BW/d. Intake varied across days (P<0.0001), but group and day did not interact (P=0.8798).

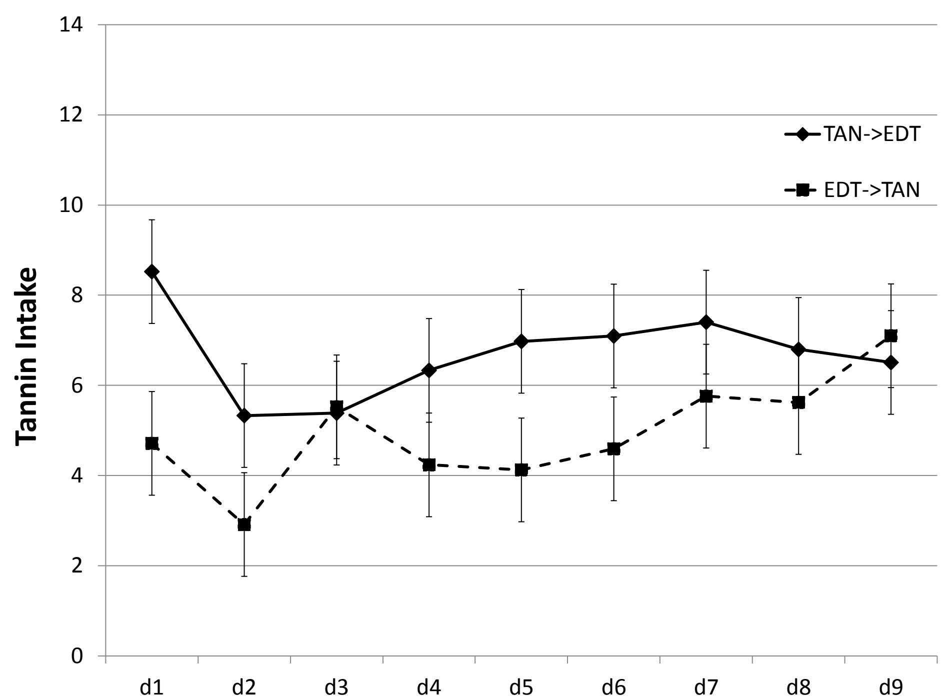

Figure 4. Intake of supplemental food containing tannins (TAN) by sheep offered foods in two sequences (TAN-->EDT or EDT→TAN) during Experiment 1. Bars are standard errors.

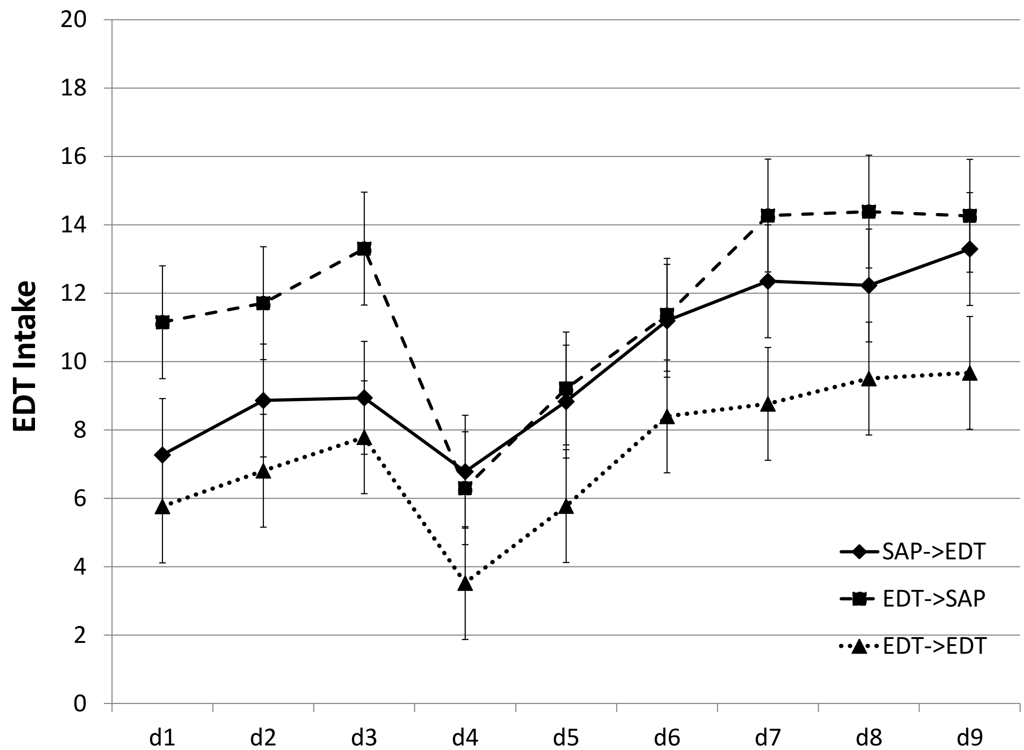

Figure 5. Intake of food containing ergotamine d tartrate (EDT) by sheep during Experiment 2. Sheep were supplemented with food containing saponins before EDT food (SAP→ EDT), with saponin after EDT (EDT→ SAP), or with only EDT (EDT→EDT). Bars are standard errors.

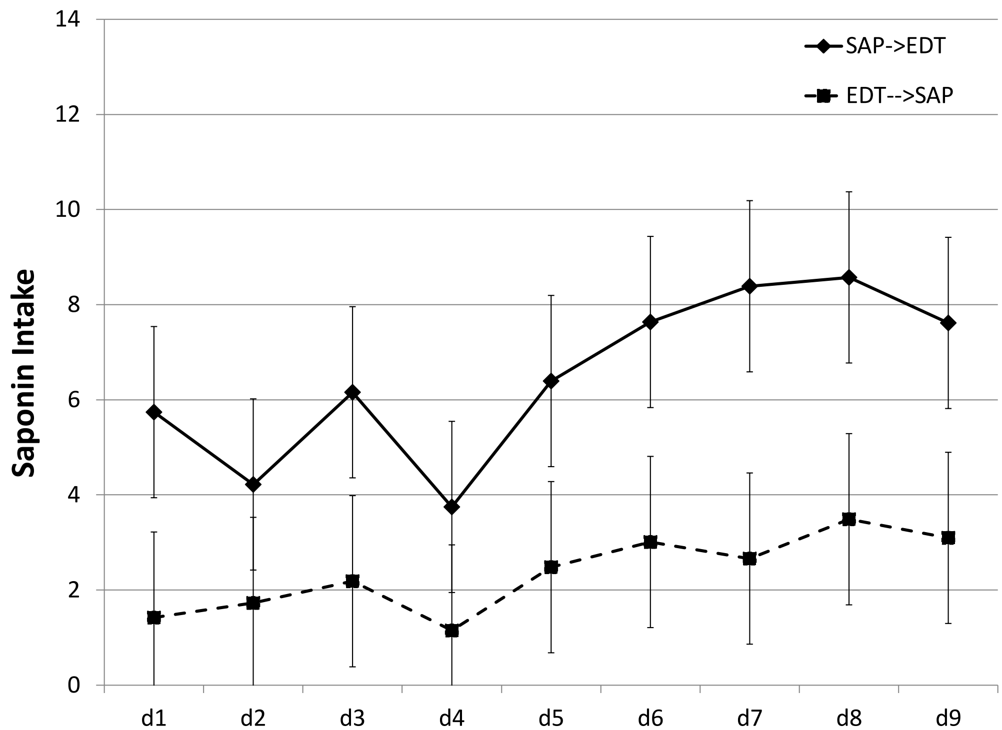

Figure 6. Intake of supplemental food containing saponins (SAP) offered foods in two sequences (SAP-->EDT or EDT→SAP) by sheep during Experiment 2. Bars are standard errors.

Discussion

Effects of Sequence on Intake of TAN, SAP, and EDT and Total Food Intake

We showed previously that supplementing sheep with tannins or saponins increases intake of food containing alkaloids (Lyman et al., 2008), but we do not know if the sequence of food ingestion affects responses to alkaloids, tannins, and saponins. Based on biochemical complementarities among these compounds, we hypothesized that the sequence in which alkaloids, tannins, and saponins are ingested would influence the ability of sheep to consume these SC. Endophyte-infected tall fescue (*Lolium arundinaceae*) contains alkaloids that are steroidal in nature. Saponins in plants such as alfalfa (*Medicago sativa*) are non-polar steroidal compounds with detergent properties and a high affinity for binding to lipids and sterols in the gastro-intestinal tract, causing their excretion in the feces (Malinow, et al., 1979). Tannins have a high affinity for binding proteins and for binding alkaloids *in vitro* (Jones and Mangan, 1977; Okuda, et al., 1982; Wong and Provenza, unpublished data).

Sequence. Lambs fed TAN→EDT or SAP→EDT did not eat more EDT compared with lambs fed EDT→TAN or EDT→SAP (Figures 1, 2). Conversely, lambs fed either TAN→EDT or SAP→EDT ate more TAN and SAP than lambs fed EDT→TAN or EDT→SAP (Figures 3, 4). Thus, the effects of sequence were more consequential for increasing intake of TAN and SAP than for increasing intake of EDT.

Total Food Intake. Sheep fed TAN and EDT ate more than sheep fed only EDT: TAN→EDT (24g/kg BW/d) = EDT→TAN (23 g food/kg BW/day) > EDT→EDT (17 g food/kg BW/d). Conversely, intake did not differ for sheep fed SAP: SAP→ EDT (14g/kg BW/d) = EDT→SAP (17 g food/kg BW/d) = EDT→EDT (16g/kg BW/d). Importantly, total food intake was higher for sheep fed TAN than for sheep fed SAP.

Sheep eat more total food and more food containing alkaloids (endophyte-infected tall fescue and reed canarygrass) when they first eat either high-tannin birdsfoot trefoil or high-saponin alfalfa; the synergistic effect is much greater for high-tannin birdsfoot trefoil than for high-saponin alfalfa (Owens et al., 2012a). The same is true for intake of a high-alkaloid food when sheep first eat food to which either tannins or saponins have been added (Owens et al., 2012b). Heifers foraging on pastures also spend much more time grazing endophyte-infected tall fescue (ergot alkaloids) when they first eat high-tannin birdsfoot trefoil or high-saponin alfalfa (Lyman et al., 2011a, 2011b). However, heifers spend much more time eating fescue when they first eat trefoil or alfalfa. Sheep also eat more endophyte-infected tall fescue when they first receive intraruminal infusions of tannins or saponins (Villalba et al., 2011).

Complementarities among diverse plant species may be enhanced or reduced depending on the timing of ingestion of the specific dietary ingredients. This is because the temporal order in which foods enter the rumen may influence interactions among different dietary chemicals and products of rumen metabolism. For instance, lambs offered SC in the sequence of tannins→ terpenes consume twice as much food as lambs offered a meal of terpenes→tannins (Mote et al., 2008). Tannins are large polar molecules that interact with other compounds as they move through the gastrointestinal tract (Kumar and Singh, 1984). Thus, consumption of tannins first increases the likelihood of interaction, and possible deactivation, of terpenes fed subsequently in a meal. In contrast, terpenes and alkaloids are small non-polar molecules, highly soluble in membranes; they are absorbed readily through the walls of the gastro-intestinal tract. When foods with terpenes or alkaloids are eaten first a sequence, the likelihood of these compounds interacting with tannins decreases. The same is true with nutrients. The sequential supply of a tannin-containing shrub (*Acacia cyanophylla*) followed by a protein-rich food substantially increases the chances of tannins interacting with proteins in the shrub, which in turn reduces ammonia formation and increases protein retention in sheep and goats (Ben Salem et al., 2005).

Collectively, these findings suggest that the bioavailability of tannins and saponins depends on the temporal sequence of forage selection when herbivores graze diverse pastures. In the current pen studies, the effect of sequence on alkaloid (EDT) intake may have been attenuated because saponins and tannins have a long residence time in the gastrointestinal tract; the retention time of quebracho tannin ranges from 48 h (free and soluble) to 72 h (bound to protein and fiber) (Silanikove et al., 1994; 1996).

We also caution that tannins and saponins are large classes of compounds with diverse structures. We used only one kind of tannin (extracted from *Aspidosperma quebracho*) and saponin (extracted from *Quillaja saponaria*). Differences in structure alter the biological activities of tannins (Clausen et al., 1990). Although saponins from *Quillaja saponaria* possess triterpenoidal saponins with structural similarity to saponins in alfalfa, the biological activity of saponin from these two sources may differ (Livingston et al., 1977; Higuchi et al., 1988; Klita et al., 1996).

Interactions among Tannins, Saponins, and Alkaloids

Plant SC play important functions in agro-ecosystems. They are vital for attracting pollinators and seed dispersers, helping plants recover from injury, protecting plants from ultraviolet radiation, and defending plants against pathogens (Rosenthal and Janzen 1979; Rosenthal and Berenbaum 1992). Moreover, the lack of SC can negatively impact plant persistence and adaptability (Asay et al. 2001). In addition, SC are increasingly recognized as important in the nutrition and health of animals (Engel, 2002; Crozier et al., 2006). Plant-derived alkaloids, tannins, terpenes and saponins have anti-parasitic properties (Hocquemiller et al., 1991; Athasianadou et al., 2001), they alleviate bloat (Waghorn, 1990), and tannins and saponins may reduce methane emissions and improve nutrient utilization in ruminants (Barry et al., 2001; Goel and Makkar, 2011). Tannins and other polyphenolic compounds can also negatively impact the viability of *E. coli* O157:H7 (Wells et al., 2005).

Plant SC limit intake through feedback mechanisms that prevent damage to animal cells, tissues and metabolic processes (Cheeke and Shull, 1985; Cheeke, 1988; Osweiler et al., 1985;

Provenza, 1995). However, herbivores can attenuate the negative impacts of single SC by eating a variety of complementary forages with different SC. In so doing, they can enhance nutrient intake while taking advantage of the beneficial properties of SC (Freeland and Janzen 1974; Provenza et al. 2003).

Our results show tannins and saponins enhanced use of ergotamine, a key alkaloid in endophyte-infected tall fescue. In a previous study, sheep fed food containing alkaloids (gramine or ergotamine) in combination with tannin- or saponin-containing foods ate more food with alkaloid than sheep offered only foods with gramine or ergotamine (Lyman et al., 2008). Our current study shows much higher intakes of ergotamine-containing food when sheep are also fed tannin food than with saponin food, which suggests the effect of tannins is more consequential at enhancing intake of ergotamine than that of saponins. These findings are consistent with studies that show sheep fed high-tannin birdsfoot trefoil for 30 min subsequently increased intake of alkaloid-containing foods (endophyte-infected tall fescue or reed canarygrass) compared with sheep fed high-saponin alfalfa for 30 min (Owens et al., 2012a). Tannins increased intake and also improved nitrogen retention when an alkaloid-containing food was supplemented with tannins (Owens et al., 2012b).

Conclusion

Historically, SC were thought of only in terms of their negative effects on food intake and production in herbivores. An overabundance of any one SC in the diet decreases intake, causes health problems, and harms profitability and production. The positive effects of SC at appropriate doses are only recently being discovered for plant and animal health (Provenza et al., 2007). Our research shows offering a variety of foods allows sheep to ingest more foods that contain SC, which can have nutrition and health benefits for animals as well as for the health of pastures and rangelands (Provenza et al., 2007). Conversely, when animals are presented with only one food in their diets, transient food aversions decrease intake even if an animal is suited to that particular nutrient or SC profile (Provenza 1996; Provenza et al., 2003). Herbivores can eat more than one SC at a time as different compounds produce different effects and are detoxified by different mechanisms within the body (Freeland and Janzen, 1974; Provenza et al., 2003). Although results from our study do not suggest that the sequence in which tannins, saponins and alkaloids were ingested increased use of an alkaloid-containing food by sheep, other studies show that the sequence of ingestion of foods containing these compounds plays a crucial role in the nutrition of animals and has implications for managing landscapes (Lyman et al., 2011a,b). Further studies are needed to extend the findings of this research for sheep and cattle foraging on pastures or rangelands.

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CHAPTER 4

INFLUENCE OF DRINKING WATER WITH TANNIN ON INTAKE OF ENDOPHYTE-INFECTED TALL FESCUE BY CATTLE

Abstract

Endophyte-infected tall fescue (TF) contains alkaloids that can adversely affect health of livestock. The effect is exacerbated when fescue is grown in monoculture. Plants such as birdsfoot trefoil contain condensed tannins which mediate the negative effects of alkaloids in TF and increase livestock intake of TF. Tannins have a high affinity for binding to the steroidal and protein-like alkaloids found in TF. Cattle and sheep eat forages with tannins and they also drink water that contains condensed tannins up to 2% of their daily food intake. Our objective was to determine if intake of TF by cattle would be increased by drinking water containing condensed tannins. We hypothesized cattle would drink water with tannins and that tannin in water would enable cattle to consume more TF than animals given only water. Cattle supplemented with tannin water drank less water each day than cattle that drank only fresh water (17.7 kg vs. 24.6 kg; $P<0.005$). They also tended to eat less TF than cattle provided fresh water (4.3kg vs. 5.1kg; $P=0.1013$). Intake of TF varied across days ($P<0.0001$), but Group did not interact with Day.

Introduction

Worldwide, landscape managers are continually looking for ways to improve livestock and crop production while also maintaining ecological integrity and reducing costs of production. Unearthing ways to sustainably feed and clothe people is important for producers increasingly challenged to meet the world's needs for food while maintaining the integrity of natural systems. Utilizing plants such as endophyte-infected tall fescue (TF) that can survive under stressful environmental extremes including drought and heavy grazing is one way to meet both environmental and economic objectives. Yet, plants such as TF often contain high amounts of secondary compounds that limit intake by herbivores and may cause health problems when eaten in too large amounts.

Endophyte-infected tall fescue contains alkaloids that can adversely affect health of livestock, especially when fescue is grown in monoculture. Plants such as birdsfoot trefoil contain condensed tannins which appear to mediate the negative effects of alkaloids in TF and increase livestock intake of TF (Lyman et al., 2008, 2011a,b; Owens et al., 2012; Villalba et al, 2011). Tannins have a high affinity for binding to the steroidal and protein-like alkaloids found in TF (Malinow et al., 1979; Jones and Mangan 1977; Okuda et al., 1982). Cattle and sheep eat forages with tannins and they also drink water that contains condensed tannins up to 2% of their daily food intake (Kronberg, 2008, 2010).

These findings suggest livestock may increase their preference for forages such as TF high in alkaloids if they are supplemented with water containing tannins, as tannins may reduce the negative physiological effects of the alkaloids. Our objective was to determine if intake of TF by cattle would be increased by drinking water containing condensed tannins. We hypothesized cattle would drink water with tannins and that tannin in water would enable cattle to consume more TF than animals given only water.

Materials and Methods

In 2006, monocultures of tall fescue (TF) (*Festuca arundinaceae, Kentucky 31 endophyte-infected*) (Rottinghaus et al., 1991) were planted at the Utah Agricultural Experiment Station Pasture Research Facility in Lewiston, UT (41’57 N. 111’52 W.). During the trials, TF 20 to 30 cm high and in a vegetative state, was harvested every morning at 0600 and transported to the Poisonous Plants Research Facility in Richmond, UT during the summer of 2009.

Trials. Sixteen fall-born calves (251kg ± 41.8kg BW) were fed the freshly cut TF from 0700 to 0830. Calves were housed indoors in individual, adjacent pens measuring approximately 4.5 x 6.09 m. Following the morning trial, 5 kg of orchard grass hay was fed to each calf. The trial lasted 19 d, with a 10-d period before the trial to allow calves to adjust to the pens and new environment and a 9-d collection period. Foods were offered in individual 68 liter containers secured within each pen. Water was offered in 95 liter buckets placed opposite each food trough. Prior to the trial, during the adjustment period, cattle were familiarized with tannin-containing water while they were fed a diet of orchard grass hay. Over a period of 10 d, cattle in the tannin group were offered water with 0.25%, 0.5%, 0.75% and 1% tannins. Tannin concentrations used for this trial were based on the work of Kronberg (2008), who found that cattle and sheep readily drink water containing tannins at concentrations up to 2% of their daily feed intake.

During the trial, cattle in one group (n=8) were offered 25 liters of water containing quebracho tannins (1% w/v), while cattle in the other group (n=8) were offered 25 liters of water without tannins. All cattle were fed 9 kg of freshly harvested TF each morning. Food and water refusals were weighed between 0830 and 0930 each day to determine the amount of tannin-containing water cattle drank when ingesting TF and if they ate more TF than cattle only drinking fresh water. Orchard grass hay refusals were not weighed, as all calves ate the entire 5 kg offered following daily trials. Procedures followed the protocols for animal care and use (IACUC protocol approval number 1372).

Chemical Composition of the Forages. Representative samples of TF were hand-harvested at 25-50 mm above ground level (grazing height) at the end of the study, placed in plastic bags covered with dry ice immediately after harvest, and transported to a freezer where they were kept at -20 ^{o}C. They were subsequently freeze dried, ground through a Wiley mill with a 1-mm screen, and analyzed for neutral detergent fiber (NDF) (all samples; Goering and Van Soest, 1970), nitrogen (all samples; Method 990.03; AOAC, 2002), and ergovaline (TF; Rottinghaus et al., 1991).

Results

Cattle supplemented with tannin in their water drank less water each day than cattle that drank only fresh water (17.7 kg vs. 24.6 kg; $P<0.005$; Figure 7). They also ate less TF than cattle provided fresh water (4.3kg vs. 5.1kg; $P=0.1013$; Figure 8). Intake of TF varied across days ($P<0.0001$), but Group did not interact with Day ($P=0.4336$; Figure 7).

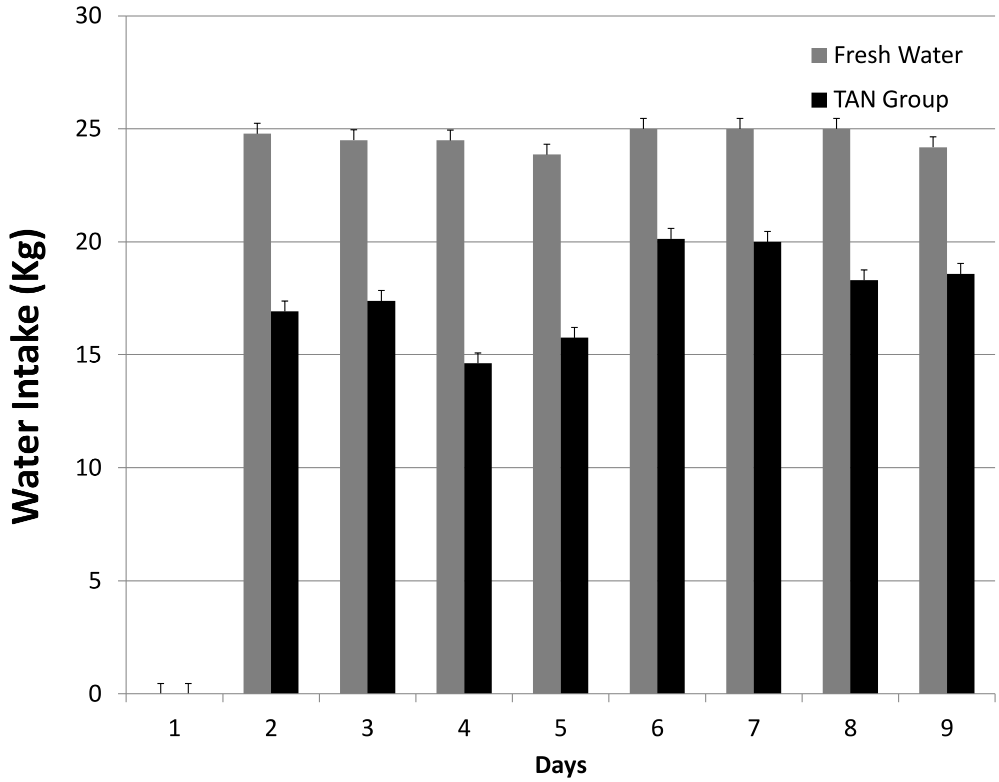

Figure 7. Intake of fresh water or water containing 1% condensed tannins by cattle. Bars are standard errors.

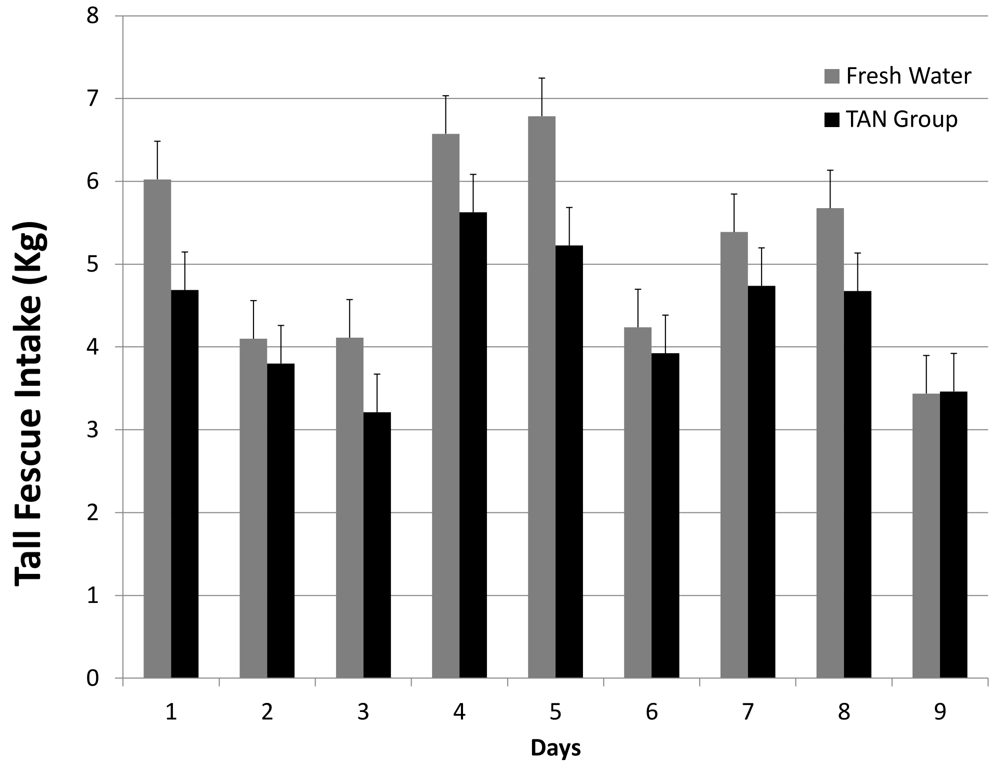

Figure 8. Intake of fresh cut Endophyte-Infected Tall Fescue by cattle offered fresh water or fresh water containing 1% tannin. Bars are standard errors.

DISCUSSION

Herbivores eat more of foods containing alkaloids when supplemented with tannins. Stable complexes form between alkaloids and tannins in the gut of the animal, inhibiting alkaloid absorption, thus enhancing intake of foods containing alkaloids (Okuda et al., 1982; Wong and Provenza, unpublished data). Tannins in forages such as birdsfoot trefoil appear to assist animals in coping with the alkaloids in tall fescue (Lyman et al., 2011a,b; Lisonbee et al., 2009; Owens et al., 2012). Lambs given intraruminal infusions of tannins increase use of high-alkaloid varieties of tall fescue relative to lambs not infused with tannins, and when offered choices among three varieties of forages containing tannins, alkaloids and saponins, lambs infused with tannins consumed much more TF than lambs not infused (Lisonbee et al., 2009; Villalba et al., 2011). Sheep eat more of food containing ergotamine d tartrate (EDT; an alkaloid found in TF) when also given food containing tannins, than sheep only offered food containing EDT (Lyman et al., 2008). These studies, and many others, suggest that supplementing cattle ingesting TF with tannins may influence their preference for TF.

In three separate studies, Kronberg (2008) found that cattle and sheep fed a high-protein diet readily drank water that contains quebracho tannins at concentrations of up to 2% of their daily food intake. In the first study, cattle maintained daily water intake when water varied in concentrations of quebracho tannins (0.125%, 0.25%, 0.5%, 1.0%, 1.5%, and 2.0% tannins). In the second study, cattle preferred tap water over water containing tannins (1%), yet they still drank some tannin water daily (14% to 70% of total daily water intake). In the third study, sheep showed no preference for fresh water over tannin water when given a choice, although daily intakes varied greatly among individuals.

Results from these studies led us to hypothesize that cattle supplemented with quebracho tannin in their water would meet their daily water requirements as well as increase their intake of TF compared with cattle not given quebracho tannin in their water. In our study, quebracho tannin significantly decreased (5 kg/d) the amount of water cattle were drank, which likely adversely impacted their intake of TF.

Dry matter and water intake are linearly related to one another. As water intake decreases, dry matter intake also decreases (MacFarlane and Howard, 1972; Silanikove, 1987). Furthermore, water restriction and dehydration reduce food intake and any agent that limits the ability of livestock to meet their water needs will adversely affect their ability to maintain food intake (Balch et al., 1953; Silanikove, 1985).

Differences in quebracho-tannin-water consumption between our study and that of Kronberg (2008) may be due in part to differences in crude protein in cattle diets. Protein concentrations may influence preference for tannin containing forages (Fernández et al., 2011). Crude protein concentration in the orchard grass hay and fresh-cut tall fescue (6-7%CP; 231 ppb ergovaline) used in this study may not have been high enough for cattle to benefit from by-pass effects due to tannin binding with protein (Kronberg, 2010 and personal communication). As a result, cattle may have formed a mild aversion to quebracho–tannin water if the tannin reduced available protein in the rumen below levels needed for rumen function.

Conclusions

Results from this study do not suggest that supplementing cattle with water containing condensed tannins increases intake of TF because tannins limit intake of water, although low protein diets may have impacted preference for water containing tannin. If cattle and other livestock could meet daily water needs while drinking tannin water, then further efforts could be made to discover the viability of tannin water influencing TF consumption.

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CHAPTER 5

INTAKE OF FOOD CONTAINING ERGOTAMINE D TARTRATE BY SHEEP OFFERED FOODS WITH TANNINS OR SAPONINS OR A CHOICE OF TANNINS AND SAPONINS

Abstract

Secondary compounds (SC) are a crucial part of plant and animal systems. They influence palatability, resistance to stress and disease, resilience and the overall health of plants and animals alike in ways that have yet to be fully understood. Various SC interact across a broad spectrum of species in ways that can be beneficial or detrimental depending on the kinds and concentrations of SC. We sought to understand how tannins and saponins affected intake by sheep of foods containing ergotamine d tartrate (EDT), one of many alkaloids in endophyte-infected tall fescue. Tannins and saponins are high molecular weight compounds that stay in the gut for many hours where they can interact with and neutralize alkaloids in the gastro-intestinal tract of animals, enabling their excretion from the body. While eating food with tannins *or* saponins increases intake of foods containing alkaloids, eating foods with tannins and saponins may attenuate these effects because tannins and saponins also interact with each other. We determined if sheep would eat more of food containing EDT when first given food containing tannin (TAN) or saponin (SAP) compared with sheep first given a choice of both TAN and SAP (CHOICE) or sheep first given food with no additional SC (CNT). Averaged across days, CHOICE (254 g) and TAN (285 g) ate similar amounts of EDT and their intakes were higher than those for SAP (200g) and CNT (170g) (P=0.0016). Thus, offering lambs a choice of TAN and SAP in combination did not decrease intake of EDT. Lambs preferred SAP over TAN, and given a

CHOICE they ate more EDT than lambs supplemented with only SAP or CNT. Results from this study suggest that SC interact in ways that can be both beneficial and detrimental, and that more research is needed to understand their various interactions.

Introduction

Plant secondary compounds (SC) limit how much of any particular food an herbivore can eat, and when eaten in large amounts, SC can decrease intake, production and health (Freeland and Janzen, 1974; Provenza, 1996; Foley et al., 1999). Interactions among SC can lead to complementary relationships such that eating a combination of foods may exceed the benefit of consuming any one food in isolation (Tilman, 1982; Provenza et al., 2003). Sheep eat more when offered three foods that contain terpenes, tannins, and oxalates than when offered foods with only one or two of these SC (Villalba et al., 2004). Sheep fed either gramine or ergotamine in combination with tannin- or saponin-containing foods had higher intakes of food than sheep offered only foods with gramine or ergotamine (Lyman et al., 2008). Sheep eat more total food and more of high-alkaloid forages such as endophyte-infected tall fescue and reed canarygrass when they first eat high-tannin birdsfoot trefoil or high-saponin alfalfa (Owens et al., 2012a). The same is true for intake of a high-alkaloid ration when sheep first eat a ration to which tannins or saponins have been added (Owens et al., 2012b).

Complementarities among SC are an important but little understood area of plant-herbivore interactions. Even less is known about how the sequences of eating foods with different SC affects foraging, though they appear to be critical (Provenza et al., 2003). Sheep eat more of foods containing tannins and terpenes when they are given the food with tannins first followed by the food with terpenes, whereas the reverse sequence does not encourage intake of either food (Mote et al., 2008). The sequence of forage ingestion also causes cattle to spend more time grazing grasses with alkaloids (endophyte-infected tall fescue and reed canarygrass) when they eat legumes containing saponins or tannins (alfalfa or birdsfoot trefoil) before grasses than when the sequence is reversed (Lyman et al., 2011 a,b). Tannins and saponins are high-molecular-weight compounds (2,000 to 4,000) that remain in the gut for many hours where they interact with many other compounds (Kumar and Singh, 1984; Min and Hart, 2003). Thus, eating food with tannins or saponins first allows these compounds to interact with other SC, such as alkaloids, as they are ingested.

Tannins and saponins also interact with one another to influence food intake. Rats eat more of a combination of foods containing tannins and saponins than of either food alone, evidently because tannins and saponins bind in the gastrointestinal tract, reducing the negative effects of both compounds on food intake (Freeland et al., 1985). While eating food with tannins or saponins increases intake of foods containing alkaloids, eating foods with tannins and saponins may attenuate these effects because tannins and saponins also interact with each other. We thus determined intake of food containing alkaloids by sheep first given food containing either tannins or saponins compared with sheep first given a choice of two foods, one with tannins and the other with saponins.

Materials and Methods

Lambs were 6 Mo old, commercial Rambouillet-Columbia-Finn-Targee crossbred lambs (40.5Kg ±4.4kg). They were acquired from the same farm, and then moved to the Green Canyon Ecology Center, Utah State University, Logan, Utah. They were penned individually with free access to trace mineralized salt blocks and fresh water. Lambs were kept outdoors, under a protective roof in individual, adjacent pens measuring 2.4 x 3.6 m. All procedures were approved by the Utah State University Institutional Animal Care and Use Committee (IACUC #1413).

Experimental Design.

Lambs were adjusted to pens and introduced to all foods and SC over a 10-d period. During the 9-d trial, groups of lambs (n=8/group) were fed a sequence of food containing ergotamine d tartrate (EDT), tannins (TAN), saponins (SAP), or no additional SC (CNT) in 2 feeding periods. During the experiment, lambs in Group 1 were offered a CHOICE (125g of TAN and 125g of SAP) for 30 min, then 1000g of EDT for 1 h, then another CHOICE for 30 min, followed lastly by EDT (1000 g) for 1 h. Lambs in Group 2 were given 250g of TAN for 30 min, then 1000g of EDT for 1 h, then 250g of TAN for 30 min, followed by 1000g of EDT for 1 h. Lambs in Groups 3 and 4 followed the same protocols as lambs in Group 2, but SAP (Group 3) or CNT (Group 4) replaced TAN. Food refusals were weighed to determine intake of EDT, TAN, and SAP by lambs in Groups 1 to 4.

Foods.

For each experiment, a basal diet of 58% ground alfalfa pellets and 38% ground beet pulp was mixed with ergotamine D tartrate (EDT) (Sigma-Aldrich Inc., St Louis, MO) at 30ppm; 2% onion powder was added to EDT to act as a flavor cue that signaled the addition of EDT to the food. The TAN food was 54% ground alfalfa, 34% ground beet pulp and 10% tannins. The SAP food contained 58% ground alfalfa, 38% ground beet pulp and 2% saponin. The CNT food contained 59% ground alfalfa, and 39% ground beet pulp. All diets were mixed with 2%

vegetable oil to reduce dust and inhalation of SC, particularly saponins, which tended to form a fine dust when mixed with food.

The kinds and concentrations of SC correspond with levels known to affect herbivores. Ergotamine at 30 ppm causes physiological changes similar to those observed in animals suffering from fescue toxicosis (Osborn et al., 1992). Tannin concentrations of 10% are in the range of Lotus spp. (Min and Hart, 2003). The condensed tannin we used, extracted from *Aspidosperma quebracho* (Tannin Corporation, Peabody, MA), is a complex of tannins, flavonoids, and other phenolic compounds (Mole and Waterman, 1987) containing approximately 85% condensed tannin (Titus and Provenza, unpublished results). Saponins at 2% decrease food intake by sheep (Burritt and Provenza, 2000). The saponin we used was extracted from *Quillaja saponaria* (Sigma-Aldrich Inc., St Louis, MO). Tannins, saponins, and ergotamine D tartrate were all fine powders, which we thoroughly mixed with the other food ingredients that were ground to 1-2 mm particle size.

Statistical Analysis.

The statistical design for the analysis of variance was a split-plot with lambs nested within group; daily measurements of food intake were the repeated measures (day) on each lamb. The response variables were the amount (g/lamb/d) of food consumed containing EDT, TAN, SAP, or CNT and the total amount of food consumed/day.

Results

Ergotamine Intakes.

Intake of EDT varied by Group (P= 0.0016) and by Period (first feeding period = 317g, second feeding period = 136g; P<0.0001), but Period did not interact with Group (P=0.4208).

Averaged across days, CHOICE (254 g) and TAN (285 g) ate similar amounts of EDT and their intakes were higher than those for SAP (200g) and CNT (170g) (Figures 9 and 10). Intake varied by day ($P<0.0001$), and Group interacted with Day ($P=0.0001$; Figure 11).

Tannin and Saponin Intakes by Feeding Periods.

Lambs ate more in the first than the second feeding periods regardless of group ($P<0.0001$): TAN ate 158g and 112g of food with tannins, SAP ate 235g and 198g of food with saponins, and CNT ate 218g and 192g of food without added SC. Group tended to interact with Period ($P=0.081$; Figure 12). Lambs in CHOICE ate less food with tannins than with saponins during both the first (93g vs. 111g) and second (41g vs. 107g) feeding periods, ($P<0.0001$).

Total Food Intakes.

Averaged across Days and Periods, groups did not differ in total food intakes ($P=0.1710$): CHOICE ate 429g, TAN ate 420g, SAP ate 416g, and CNT ate 374g. Total food intakes varied across days ($P<0.0001$), but Group did not interact with Day ($P=0.1125$) or Period ($P=0.6041$).

Feeding Periods.

Averaged across groups, sheep ate more of all foods during the first than during the second period. Comparing periods 1 and 2, sheep ate 318g vs. 136g of EDT, 158g vs. 112g of TAN, 235g vs. 198g of SAP, and 218g vs. 192g of CNT. Sheep given a CHOICE ate 93g vs. 41g of TAN and 111g vs. 107g of SAP.

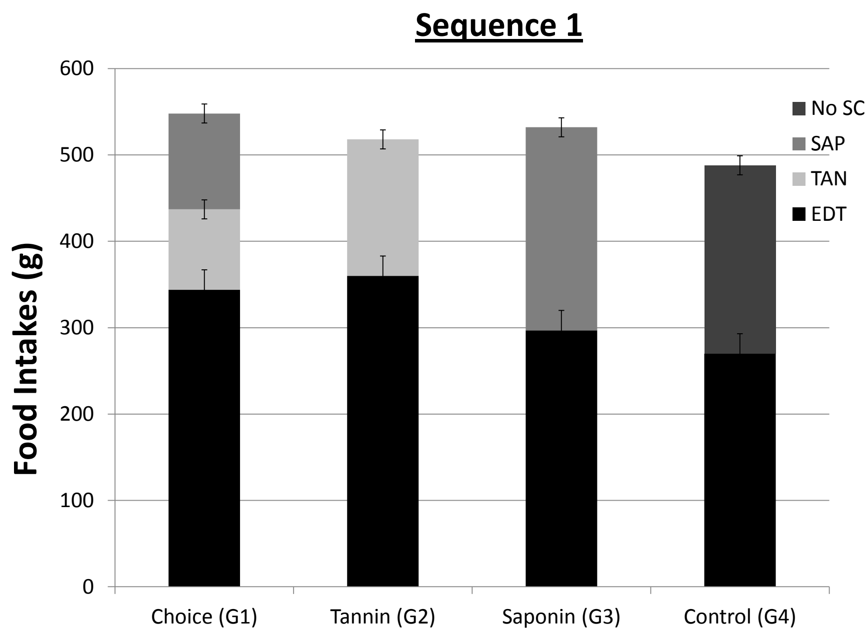

Figure 9. Intake of food containing ergotamine d tartrate (EDT), tannin (TAN), saponin (SAP) or no additional SC (CNT) by sheep during Feeding Period 1. Sheep were supplemented with a CHOICE, TAN, SAP, or CNT before EDT. Bars are standard errors.

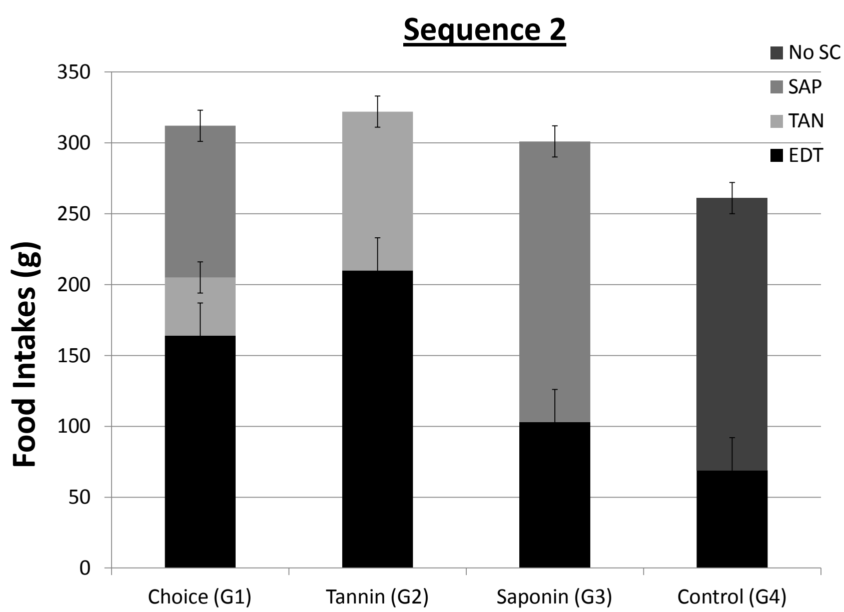

Figure 10. Intake of food containing ergotamine d tartrate (EDT), tannin (TAN), saponin (SAP) or no additional SC (CNT) by sheep during Feeding Period 2. Sheep were supplemented with a CHOICE, TAN, SAP, or CNT before EDT. Bars are standard errors.

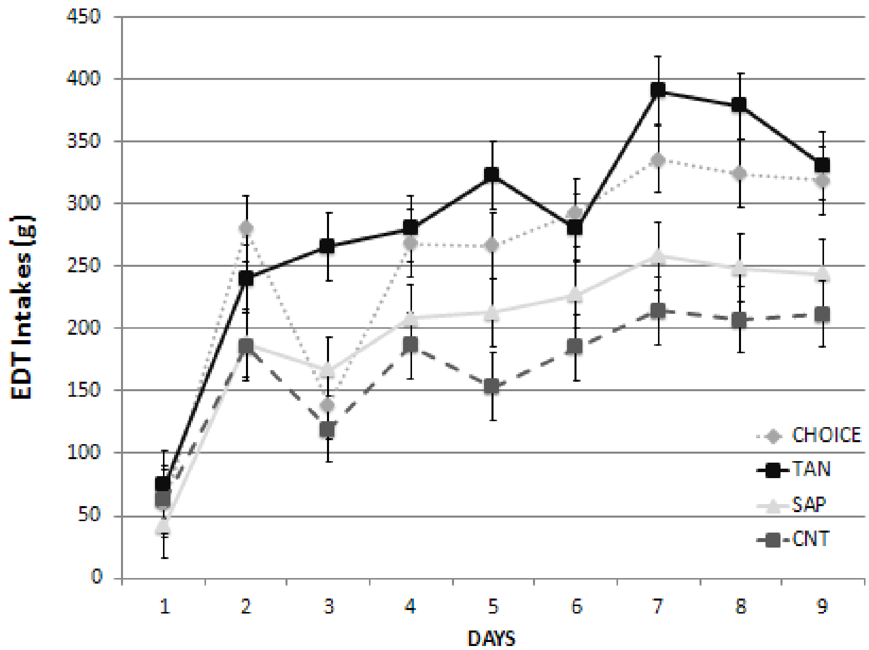

Figure 11. Intake of food containing EDT by sheep across days. Bars are standard errors.

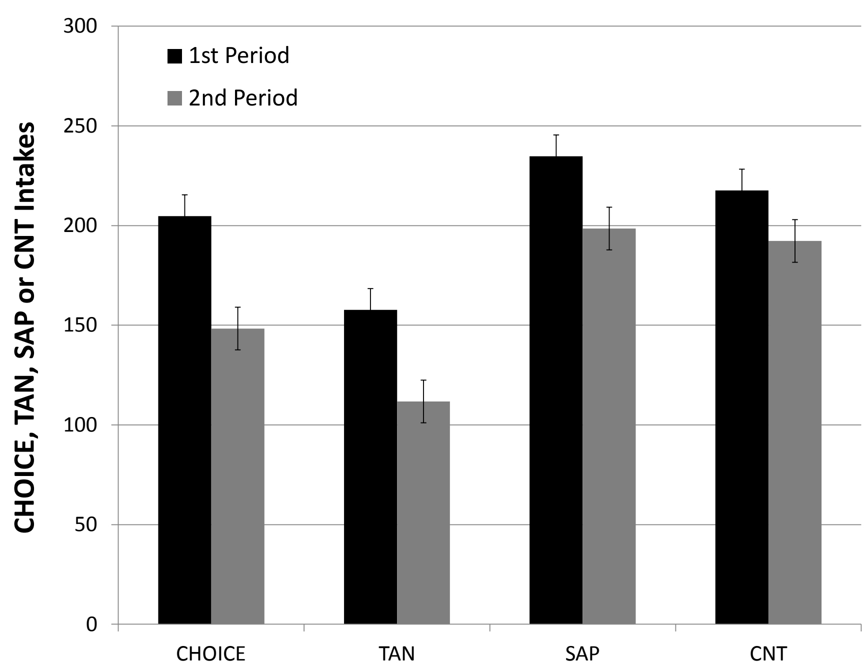

Figure12. Intake of food containing tannin (TAN), saponin (SAP) a choice of TAN and SAP (CHOICE) or no additional SC (CNT) by sheep during Feeding Period 1 and 2. Bars are standard errors.

Discussion

We determined if sheep would eat more of food containing EDT when first given food containing tannin (TAN) or saponin (SAP) compared with sheep first given a choice of both TAN and SAP (CHOICE) or sheep first given food with no additional SC (CNT). Averaged across days, CHOICE (254 g) and TAN (285 g) ate similar amounts of EDT and their intakes were higher than those for SAP (200g) and CNT (170g) (P=0.0016). Thus, offering lambs a CHOICE of TAN and SAP in combination did not decrease intake of EDT. Lambs preferred SAP over TAN, and given a CHOICE they ate more EDT than lambs supplemented with only SAP or CNT. Lambs supplemented with food containing no additional SC (CNT) had the lowest intakes of EDT. This is consistent with other studies suggesting that EDT intake is positively influenced by supplementation with tannins or saponins (Lyman et al., 2008, 2011, a,b).

Effects of TAN and SAP on EDT Intakes.

Supplementation with TAN enhanced intake of EDT compared to supplementation with SAP. Lambs fed food with tannins (TAN and CHOICE) ate more EDT than groups that were not given any food with tannins (SAP and CNT). Conversely, saponins did not greatly influence intake of EDT, although other studies suggest saponins increase preference for food containing EDT or other alkaloids (Lyman, et al., 2008; 2011a,b; Owens et al., 2012a) Lambs ate more SAP than TAN first in the sequence, which may have influenced subsequent intake of EDT as lambs may have been less hungry when presented with EDT after SAP than TAN.

This suggests that even a small amount of TAN was more influential in enhancing EDT intake than either SAP or CNT alone. Sheep fed small amounts of high-tannin foods (birdsfoot trefoil) subsequently greatly increased intake of alkaloid-containing foods (endophyte-infected tall fescue or reed canarygrass) much more compared with sheep fed high-saponin alfalfa for 30 min (Owens et al., 2012a). Furthermore, tannins increase intake and improve nitrogen retention when an alkaloid-containing food is supplemented with tannins (Owens et al., 2012b). We hypothesize that the increases in EDT intake are due to biochemical interactions with tannins in the gut. Tannins are large polar molecules that interact with other compounds as they move slowly through the gastrointestinal tract (Kumar and Singh, 1984). Tannins have a high affinity for binding proteins and for binding alkaloids *in vitro* (Jones and Mangan, 1977; Okuda, et al., 1982; Wong and Provenza, unpublished data).

Tannin and Saponin Intakes.

Sheep showed strong preference for SAP and CNT over TAN diets. Though tannins increase preference for many other foods containing SC, high amounts of tannins can suppress intake (Makkar, 2003). Lambs supplemented with high-tannin birdsfoot trefoil (BFT) eat much more endophyte-infected tall fescue (containing EDT) than lambs supplemented with high saponin alfalfa (ALF) or unsupplemented animals; the same lambs eat much less BFT than ALF, yet were stimulated to eat TF to a greater degree by BFT than by ALF (Owens et al., 2012ab). Thus, while no preference for TAN occurred during this study, a small amount of tannins might be more effective at increasing intake of alkaloids than a higher dose, and thus we hypothesize lambs ate enough TAN to neutralize EDT, but not enough to demonstrate a preference or decrease overall appetite

Choice Effect.

Sheep offered a CHOICE or TAN first had higher intakes of EDT than sheep fed only SAP or CNT first. Tannins and saponins interact with one another to influence grazing behavior, and giving animals a choice of foods containing tannin or saponin first in the feeding period reduced subsequent intake of EDT. Rats eat more of a combination of foods containing tannins and saponins, than either food alone, because tannins and saponins bind in the gastrointestinal tract, reducing the negative effects of both components (Freeland et al., 1985). Goats increase intake when shrubs contain a combination of tannins and saponins relative to animals offered single shrubs (Rogosic et al., 2006). Likewise, sheep with parasites eat more of foods containing tannins and saponins when offered a choice, yet the combination of tannin and saponin foods was less effective at reducing parasitic loads than either SC food alone; tannins and saponins bind in the gastrointestinal tract, evidently reducing the anti-parasitic activity of both compounds (Villalba, et al., unpublished data). Thus, animals may eat more food when given a diet containing multiple SC, yet due to their many complex interactions, the medicinal effects of multiple SC may be less than with a single SC on parasites.

Feeding Period.

All sheep ate the most during the first feeding period, regardless of what foods were offered. This was most likely due to sheep being previously fasted overnight. The temporal order in which foods enter the rumen may influence interactions among different dietary chemicals. For instance, lambs offered SC in the sequence of tannins followed by terpenes consume twice as much food as lambs offered a meal of terpenes followed by a meal of tannins (Mote et al., 2008). Tannins are large polar molecules that interact with other compounds as they move slowly through the gastrointestinal tract (Kumar and Singh, 1984). Thus, consumption of tannins first increases the likelihood of interaction, and possible deactivation, of terpenes fed subsequently in a meal. In contrast, terpenes and alkaloids are small non-polar molecules, highly soluble in membranes; they are absorbed readily through the walls of the gastro-intestinal tract.

Conversely, when a food with rapidly absorbed terpenes or alkaloids is eaten first in a sequence, these compounds are less likely to interact with tannins. First eating a shrub with tannins (*Acacia cyanophylla*) followed by eating a protein-rich food substantially increases the chances of protein interacting with tannins, which reduces ammonia formation and increases protein retention in sheep and goats (Ben Salem et al., 2005).

Conclusion

Monocultures are not always ideal for intensively managed pastures, due to their seasonality, susceptibility to pests and monotony of primary and secondary compounds. Furthermore, an overabundance of any one primary or secondary compound in animal diets will decrease intake, cause health problems, and harm overall profitability and production. Conversely, mixtures of plant species enhance productivity of herbivores while decreasing reliance on herbicides and insecticides to maintain plant resilience (Provenza and Villalba, 2006, 2010). Findings from this and past studies suggest that forage complementarities and sequences facilitate intake of foods containing SC, undoubtedly due to complex interactions among secondary compounds. Our findings also suggest that multiple SC interact in a myriad of ways, which may not always be beneficial to animal intake. SC can complement one another to increase herbivore’s intake of unpalatable plant species and foods. Obviously, more experimental analyses are necessary to assess the specific physiological and behavioral effects of interactions among secondary compounds, and to better understand higher-order interactions among these compounds in various forages (Provenza et al., 2003).

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CHAPTER 6

CONCLUSIONS AND IMPLICATIONS:

FOOD COMPLEMENTARITIES, SYNERGYS, AND SEQUENCES

Overview of My Research

In their efforts to describe the defensive roles of secondary compounds (SC) in plants, researchers have not considered the possible health benefits of these compounds in appropriate dosages (Engel, 2002). Everything depends on the dose: PCs and SCs at too high concentrations can be toxic, while at lower concentrations they can both have health benefits (Provenza and Villalba, 2006). Likewise, in our haste to increase the palatability of monoculture pasture species, researchers have selected for low concentrations of compounds such as alkaloids (reed canary grass and endophyte-infected tall fescue), tannins (birdsfoot trefoil), and saponins (alfalfa), not appreciating that these compounds in proper mixtures may actually benefit animals. To understand the effects of tannins and saponins on alkaloid toxicity we must first understand their interactions under controlled conditions in pens and pastures.

My objective was to better understand how livestock foraging behavior is influenced by ingesting tannins, saponins, and alkaloids, in different combinations and sequences. My research was based on hypotheses regarding how these compounds interact with each other to influence food selection and how the sequence of ingesting foods with SC influences preference (Freeland and Janzen, 1974). Research suggests that tannins and saponins interact with and bind to compounds such as alkaloids causing their excretion in the feces (Malinow et al., 1979; Jones and Mangan 1977; Okuda et al., 1982). Thus, I hypothesized that supplementing sheep or cattle eating food with alkaloids with food containing tannins or saponins would enhance intake of food with alkaloids by neutralizing alkaloids. I further hypothesized that the sequence in which these compounds and foods are eaten may further influence the likelihood of SC interactions and subsequent animal foraging behavior (Lyman et al., 2010; Meuret, 1994; Mote et al., 2008).

I addressed four questions: 1) Is intake of endophyte infected tall fescue (alkaloids) influenced by the sequence in which cattle graze birdsfoot trefoil (tannins), alfalfa (saponins), and tall fescue? 2) Is intake of food with alkaloids influenced by the sequence in which sheep are supplemented with foods containing tannins or saponins? 3) Is intake of endophyte-infected tall fescue by cattle influenced by supplementation with water containing tannin? 4) Is intake of food containing ergotamine d tartrate (alkaloid) influenced by supplementation with food containing tannin, saponin, no SC, or a choice of both tannin and saponin?

With regard to the first question, I found that cattle grazed more frequently on endophyte-infected tall fescue (TF) pastures when first allowed to graze on birdsfoot trefoil (tannin) or alfalfa (saponin) pastures, than when grazing in the reverse sequence. I hypothesize these responses were due to interactions among SC as well as primary compounds, i.e. tannins and saponins binding and enhancing alkaloid excretion and nitrogen enhancing intake of fiber, which enabled cattle to more effectively graze TF. With regard to question 2, I found that sheep ate more food with ergotamine d tartrate (EDT) when also supplemented with food containing either tannin or saponin, than sheep given only EDT food, regardless of sequence. My third study showed that adding quebracho tannin to the water of cattle eating TF did not enhance intake of TF. The addition of quebracho tannin (1%) to drinking water markedly decreased water intake, which negatively influenced intake of TF. Lastly, I found that supplementing sheep with a choice of foods containing tannin or saponin did not further enhance intake of food containing EDT, compared to sheep supplemented only with food containing tannins, possibly due to interactions among tannins and saponins. Intake of EDT by sheep given a choice remained higher than intake of EDT by sheep given only food with saponin or no additional SC, which suggests that tannins are more effective at neutralizing EDT than saponins.

Plants that can thrive in extreme climates or landscapes often contain high levels of SC, which can limit forage intake by animals. The hypothesis that SC may positively interact with one another and that herbivores are able to distinguish these relationships and use them to their benefit is not often considered. Yet, results of my studies suggest it is possible to use different combinations of SC to benefit livestock, ecosystems, and producers.

Complementarities among Primary and Secondary Compounds in Foods

Plants and herbivores interact with one another through a complex and subtle network of interrelationships mediated by SC, formerly thought to be waste products of plant metabolism (Rosenthal and Berenbaum, 1992). Historically, ecologists and agronomists emphasized the protective (defensive) roles of plant secondary compounds in plant-herbivore interactions. Where once we thought only poisonous plants contain compounds that were toxic, there is increasing awareness that all plants contain secondary compounds (Provenza et al., 2003).

We are beginning to appreciate how interactions among primary compounds (PC) and SC can lead to complementary relationships among forages, such that eating a combination of foods and compounds may exceed the benefit of consuming any one food in isolation (Tilman, 1982). We little appreciate how the sequence in which foods differing in PC and SC are ingested may affect the ability of compounds to interact within the animal, and also prove useful for animal health and grazing practices. Food complementarities have applications in human diets as well, especially when considering the nutritional and medicinal value of foods.

In the following, I discuss complementary interactions among PC and SC in foods; how the sequence of food ingestion influences interactions among chemical compounds and foraging behavior; food synergy in human diets; and applications of complementary foods and sequence in managing livestock and landscapes. We conclude by discussing practical implications and needs for future research. The various interactions among plant compounds and the animals that ingest them may have large implications to our management of natural resources. It is further evident that interactions among plant compounds, both primary and secondary, influence the health and behavior of grazing animals, humans, and the landscapes they inhabit. Understanding these interactions is crucial to understanding the complex links between us, animals, the foods we eat and the health of landscapes we rely on.

Animals can meet their needs for PC and tolerate higher total intake of SC when they can choose from a variety of plants (Provenza and Villalba, 2006). Herbivores seldom consume enough primary or SC to result in poisoning because they regulate intake of foods as a consequence of the post-ingestive feedback from PC and SC in foods (Provenza, 1995, 1996; Foley et al., 1999). For instance, oral gavages of PC and SC cause dose-dependent decreases in intake of foods that contain the SC (Villalba and Provenza, 1999; Wang and Provenza, 1997). Herbivores are able to eat more foods with different kinds of SC because they affect the body in different ways and they are detoxified by different mechanisms (Freeland and Janzen, 1974). Furthermore, a diverse intake of SC can create complimentary interactions among SC, which in turn can reduce susceptibility to toxic doses. At the most simple levels, supplemental energy and protein enhance the abilities of animals to ingest forages high in SC (Villalba et al., 2002a,b;

Provenza et al., 2003). Both PC and secondary SC in too great amounts can be toxic, whereas in appropriate amounts they interact collectively to benefit both nutrition and health (Provenza and Villalba, 2006, 2010).

Secondary compounds in plants limit intake below needs for nutrients such as energy and protein, which causes herbivores to eat a variety of plant species that contain different kinds of SC (Freeland and Janzen, 1974). Complementarities among plant species and their chemical components may enhance forage intake and digestibility, as well as increase neutralization of potentially toxic compounds. Lambs eat more of grasses high in alkaloids when they are also offered alfalfa containing saponins or birdsfoot trefoil containing tannins (Owens et al., 2012ab). Rats eat more of a combination of foods containing tannins and saponins, evidently because tannins and saponins chelate in the intestinal tract, reducing the negative effects of both components (Freeland et al., 1985). When lambs can choose between foods that contain either amygdalin or lithium chloride, they eat more than lambs offered a food that contains only one of these compounds; the same is true with nitrate and oxalate (Burritt and Provenza, 2000). Sheep also eat more when offered three foods that contain terpenes, tannins, and oxalates than when offered foods with only one or two of these SC (Villalba et al., 2004). Lambs given intraruminal infusions of tannins or saponins increase use of endophyte-infected tall fescue (with high levels of alkaloids) relative to lambs not infused with tannins or saponins (Lisonbee et al., 2009; Villalba et al., 2010). Sheep eat more of alkaloid containing foods when also supplemented with foods high in tannins or saponins (Lyman et al., 2008).

Enhanced intake of alkaloids with supplementation of tannins or saponins may occur because stable complexes form between alkaloids and tannins (Okuda et al., 1982; Wong and Provenza, unpublished data), and because alkaloids bind to saponins in the gastro-intestinal tract causing their excretion in the feces (Malinow et al., 1979). Other research suggests that tannins alone bind readily to a wide array of chemical groups and may potentially inactivate secondary compounds (Okuda et al., 1982). Lambs eat more when allowed to ingest tannin- and terpene-containing foods than when offered either food individually (Mote et al., 2008).

Other SC may have complimentary binding effects as well. Brushtail possums (*Trichosurus vulpecula*) that can select from two diets containing phenolics and terpenes eat more food than when they consume diets containing only one of these toxins (Dearing and Cork, 1999), with the same being true in principle with squirrels (*Sciurus niger and S. carolinensis*) (Schmidt et al., 1998). Mule deer (*Odocoileus hemionus*) eat more when offered both sagebrush and juniper (12.3 g/kg BW), plants that contain different terpenes, than when they are offered only sagebrush (4.2 g/kg BW) or juniper (7.8 g/kg BW) (Smith, 1959). Complementarities among SC assists herbivores in utilizing *all* plant species, with the potential to better maintain healthy animals and bio-diverse landscapes (Provenza, 2008).

Sequence of Food Ingestion Influences Foraging Behavior

When researchers began studying shepherding practices in France, they were astonished by the productivity of cattle, sheep, and goats in rugged landscapes. How do shepherds achieve such high levels of performance for meat and dairy animals? They do so by moving animals in grazing circuits that offer animals a smorgasbord of plants (Meuret et al., 1994). Daily meals are composed of various courses that differ in duration, including a brief appetizer phase of abundant but not highly preferred plants; a main course of plants of moderate abundance and preference; a booster phase of highly preferred plants for added diversity; and a dessert phase of palatable plants that complement previously eaten forages. Daily grazing circuits enable each individual to select what it needs from a range of plants that vary in primary and secondary compounds. Creating menus of various courses is how herders stimulate appetites of individuals, which enhances the nutrition, health, and overall production of the flock.

The sequence in which foods are ingested can dramatically influence the ability of herbivores to maintain satisfactory intake of PC as well as tolerate high levels of SC. Sheep eat more when foods with SC are offered in the morning followed by a limited amount of nutritious food in the afternoon (Papachristou et al., 2007). They also eat more of foods containing tannins and terpenes when they are given the food with tannins first followed by the food with terpenes, whereas the reverse sequence does not encourage intake of either food (Mote et al., 2008). Tannins remain in the gut up to 72 hours (Silanikove et al., 1994, 1996), where they can then interact with terpenes, whereas terpenes are highly soluble compounds absorbed through the gastrointestinal tract and eliminated quickly from the body (Foley and McArthur, 1994). Thus, eating food with tannins first allows the tannin compounds to interact with other chemical compounds as they are ingested. These findings are consistent with landscape-level studies that show ewes with a high preference for sagebrush, a shrub high in terpenes, also consume more bitterbrush, a shrub high in tannins, compared with ewes that have a lower preference for sagebrush (Seefeldt, 2005), as well as observations of herders in France who use meal sequences to stimulate intake of livestock (Meuret et al., 1994).

Cattle graze more high-alkaloid varieties of tall fescue and reed canarygrass when they first graze high tannin varieties of birdsfoot trefoil or high saponin varieties of alfalfa (Lyman et al., 2010). This effect is most pronounced for cattle grazing tall fescue which is much higher in alkaloids that deter grazing than reed canarygrass. In the studies of Lyman et al. (2011), sequence of forage ingestion markedly influenced the percentage of time cattle grazed tall fescue: use of fescue decreased from 40% to 15% when cattle first grazed tall fescue for 30 min followed by birdsfoot trefoil or alfalfa for 60 min. However, when the sequence was reversed, cattle actively foraged on both legumes and fescue throughout a 90-min meal.

The sequence of ingesting PC also has important implications for grazing behavior. Nitrogen in legumes and shrubs can increase intake of grass. Nitrogen increases intake of more poorly digestible fiber, especially in dormant grasses, by increasing rates of microbial decomposition in the gut. Protein supplementation increases intake of fiber (Van Soest, 1994), and meal size and length are larger in dairy cows fed a supplement before eating roughage than when the roughage is fed before the supplement (Morita et al., 1996). Sheep prefer to eat legumes in the morning and grass in the afternoon, which is likely due to interactions involving both primary and secondary compounds (Rutter, 2006).

Food Synergies in Human Diets

While food processing has greatly reduced their presence and concentrations, SC still abound when human diets contain fresh fruits and vegetables. Every variety of vegetable and fruit contains vast arrays of PC and SC that provide nutritional and medicinal benefits. For optimum health, some suggest people should consume at least five 80 gram portions of fruit and vegetables every day, as our bodies need the PC and SC these foods contain (Williams, 1995). Consumption of fruit and vegetables is associated with reduced risk of cancer, cardiovascular disease, diabetes, Alzheimer disease and a wide spectrum of other health related problems (Temple, 2000; Willett, 1994; Liu et al., 2000). Consuming plant-based foods with significant amounts of SC can provide health benefits beyond normal nutrition and assist in the defense against chronic diseases (Liu, 2003). Furthermore, the combinations in which individual foods are eaten may enhance the potency, absorption, and biological activity of the PC and SC these foods contain (Magee, 2007).

The combinations and sequences in which we eat foods, particularly fruits and vegetables, are important to the digestion and absorption of their chemical components. Prostate tumors grow much less in rats fed tomatoes and broccoli together than in rats that eat diets containing broccoli or tomatoes alone. Moreover, the combination of whole foods is better than isolated cancer-fighting substances from tomatoes or broccoli (Canene-Adams et al., 2007). People who eat 2 1/2 tablespoons of avocado in combination with a lettuce, carrot, and spinach salad absorb 8.3 times more alpha-carotene, 13.6 times more beta-carotene, and 4.3 times more lutein than those who eat only salad (Unlu et al., 2005). People also absorb nearly 4.5 times more lycopene from tomatoes when they eat 150 grams of avocado along with 300 grams of tomato salsa (Unlu et al., 2005). The combination of phytochemicals quercetin (found in apples, onions and berries) and catechin (found in green tea, apples, purple grapes, and grape juice) inhibit platelet clumping more so than either compound alone, while the phytochemicals cambene and indole 3-carbinol (found in cruciferous vegetables) better protect rats against liver cancer than either compound alone (Magee, 2007). Vitamin C, found in citrus fruits and other foods, enhances absorption of iron found in leafy green vegetables (Lynch and Cook, 1980). Corn and beans are staples in the diets of many American cultures and a major source of calories. Each is inadequate in specific essential amino acids, yet when eaten in combination, corn and beans meet the needs for amino acids and are a great source of energy.

People add spices to flavor foods, rarely appreciating their nutritional and medicinal values. Spices benefit digestive process (Viuda-Martos, et al., 2011) in several ways, including increasing the secretion: of saliva and gastric juices (Tapsell et al., 2006), of biliary acids, which play a fundamental role in digesting and absorbing fatty acids (Bhat et al., 1984), and of digestive enzymes such as lipase, amylase, trypsin, and chemotrypsin, all important in digestion (Tapsell et al., 2006). Spices like turmeric reduce irritation to the stomach and the pungency of food by increasing the mucin content of gastric juices. Spices such as ginger, mint, ajowan, cumin, fennel, coriander, and garlic are used commercially as digestive stimulants and in home remedies for digestive disorders including flatulence, indigestion, and intestinal disorders (Srinivasan, 2005). Marjoram added to salad increases the antioxidant capacity of the salad by 200 percent, illustrating the benefits of adding herbs and spices to flavor foods (Ninfali et al., 2005). Thus, while human cultures use spices to increase the palatability and "flavor" of foods, these studies suggest our desire for spices is also linked to enhancedfood digestion.

Nutritional and medicinal effects from foods cannot always be attributed to the activity of only one or two specific compounds in a food. For example, the risk of cancer is inversely correlated with the consumption of green and yellow vegetables and fruit, most of which contain beta-carotene, thought to be the active against cancer cells. However, when clinical trials provide patients with skin cancer a beta-carotene supplement, the incidence of the cancer was unchanged (Hennekens et al., 1996). In another study, patients gained no benefit from beta-carotene supplementation for incidence of lung cancer (Omenn et al., 1996). Eating the skin with an apple nearly doubles the antioxidant capacity of apples, due primarily to greater phenolics and flavonoids in the skin (Liu, et al., 2001). Apples eaten with the skin also better inhibit proliferation of human colon and hepatic cancer cell lines (Wolfe et al., 2003; Liu et al., 2001). The same is likely to be true for men who eat whole tomatoes, as opposed to taking lycopene for prostate health. Rats that consume tomatoes are 26 percent less likely to die of cancer when exposed to prostate-causing-chemical than rats that consume lycopene as a placebo (Boileau et al., 2003). The beneficial effects on colon and liver cancer (colon and liver reductase activity) are greater when people eat whole, fresh broccoli with a full complement of glucosinolates than when one of the glucosinolates in broccoli (glucoraphanin) is processed into the anti-cancer compound sulphoraphane (Jeffery and Araya, 2009). Pomegranate juice has much higher antioxidant activity, and greater ability to inhibit proliferation of oral, colon, and prostate tumor cell lines, than do any of its polyphenolic constituents including punicalagin, ellagitannin, and ellagic acid alone (Seeram et al., 2005). Eating pomegranate peels increases plasma ά-tocopherol in calves, probably because polyphenols protect ά-tocopherol from oxidation during digestion; ά-tocopherol is the form of vitamin E preferentially absorbed and accumulated in humans (Shabtay et al., 2008). Thus, the combination of phytochemicals in fruit and vegetables is responsible for their health benefits, not one compound alone.

Importance of Complimentarities in Livestock and Land Management

Agronomists and ecologists alike have come to view SC as defenses against herbivory because SC limit food intake. However, herbivores naturally regulate their intake of SC to ingest adequate levels of nutrients and avoid toxicosis, by eating a variety of foods (Freeland and Janzen, 1974; Provenza et al., 2003). Variety enables individuality and increases the likelihood of providing cells with the vast arrays of primary and secondary compounds essential for their nutrition and health. Conversely, monocultures of plants high in SC, produced through inappropriate grazing practices or genetically engineered into plants, can create vicious cycles that escalate to the detriment of soil, plants, herbivores and people (Provenza et al. 2008).

Complementary foods and sequence in animal grazing systems may prove fundamental in the upcoming transition from high fossil fuel inputs to more sustainable alternatives in animal and land management. Using Nature's abundance of food and compound complementarities will help to increase forage intake by livestock and benefit the health of plants, animals and people, while decreasing our reliance on chemical enhancers to grow and manage foods (Provenza, 2008). Furthermore, the use of complimentary compounds has implications for managing unpalatable or weedy forages with livestock, reducing the need for medicinal supplements to maintain the health and well-being of animals and people, and could potentially change the highly intensive and costly ways in which we currently manage landscapes, livestock and human diets.

Attempts by land managers to control the encroachment of weedy plant species costs huge economic inputs every year. Invasive and noxious weeds are often high in SC and other grazing deterrents that decrease the palatability and use of these plants to livestock. Thus, grazing encourages the growth of weedy species while decreasing the abundance of nutritious and palatable ones (Provenza et al., 2003). Yet, the use of complimentary SC could, in fact, turn weeds into viable food sources for livestock, by inhibiting the toxic effects of SC while continuing to accommodate animal nutrient needs. For example, cattle grazing sericea lespedeza (*Lespedeza cuneata*), a problematic legume in the Southwest with high concentrations of condensed tannins, had higher ADG and increased their intake of this noxious weed by 30% when supplemented with polyethylene glycol (PEG), a polymer that neutralizes the effects of tannins (Mantz, et al., 2009). Supplementing with PEG, allowed cattle to tolerate higher levels of tannins, and provided a new alternative for controlling serecia lespedeza.

Complimentary foods and sequence also has implications for grazing management. Traditional herders in France have used meal sequences for generations to stimulate the intake of forages by livestock (Meuret et al., 1994). By using an empirical understanding of complementarities among forages and landscape diversity herders were able to stimulate food intake and more fully use the range of plants available by herding in grazing circuits (Meuret 2010). A typical grazing circuit includes various phases, all designed to stimulate the flocks' appetite and to enhance use of all of the forage resources in an area. To do so, meals include a moderation phase, which provides sheep access to plants that are abundant but not highly preferred to calm a hungry flock; the next phase is a main course for the bulk of the meal with plants of moderate abundance and preference; then comes a booster phase of highly preferred plants for added diversity; and finally a dessert phase of palatable plants that complement previously eaten forages. Daily grazing circuits are designed to stimulate and satisfy an animal's appetite for different nutrients, and they enable animals to maximize intake of nutrients and regulate intake of different secondary compounds. While the idea that a variety of foods increases "foraging motivation" may seem counter intuitive, to French herders it is the essence of how they stimulate a flock's appetite throughout a grazing circuit.

Combinations of SC may also help reduce health problems such as bloat or internal parasites, especially if animals learn to self-medicate on diverse mixtures of plants. Domestic animals self-medicate to alleviate illness. Sheep fed acid-producing substrates such as grain prefer sodium bicarbonate, which attenuates acidosis (Phy and Provenza, 1998). Sheep fed high-tannin foods increase intake of polyethylene glycol (PEG), which binds to tannins alleviating their aversive effects (Villalba and Provenza, 2002b). Sheep also learn to associate three illness-inducing substances (grain as well as tannins and oxalic acid mixed in foods) with compounds (sodium bentonite, PEG and dicalcium phosphate, respectively) that lead to recovery from those illnesses (Villalba et al., 2006b). Thus, animals are able to use foods in their environment to alleviate illness, or digestive upset.

More specifically, foods with SC can be used by animals to mediate differing states of illness. Condensed tannins in plants such as birdsfoot trefoil and sainfoin have the potential to reduce the incidence of bloat, as they reduce microbial activities, polysaccharide slime and gas production in the rumen (Min et al., 2005). Daily tannin supplementation to steers grazing bloat-inducing pastures improves animal performance and minimizes bloat frequency on animals (Min et al., 2006). Sheep learn to avoid foods associated with rumen distension (i.e. bloat) and to prefer foods eaten during relief from distension, thus suggesting that animals can and will self-medicate for bloat when given the opportunity (Villalba et al., 2009). Parasitized lambs in confinement ingest enough tannin to reduce helminthoses, even when tannins are mixed in grape pomace which provides no nutritional benefit (Lisonbee, 2008). Sheep with parasite burdens manifest greater preferences for a tannin-containing food than non-parasitized sheep, yet lost the preference when the infection was terminated by dosing with ivermectin (Villalba et al., 2009). Other studies suggest that alkaloids and terpenes also have anthelmintic effects (Kayser et al., 2003, Hocquemiller et al., 1991). When offered a mix of plants with different SC, herbivores may better meet their needs for nutrients, mediate bloat, and reduce internal parasites as a variety of SC may deliver higher total doses of medicines with multiple actions to influence a broader array of animal health problems (Villalba and Provenza 2007).

Practical Implications and Needs for Research

Worldwide, landscape managers are constantly looking for ways to improve production as well as ecological integrity. Unearthing ways to sustainably feed and clothe humanity is an important factor for producers, who will increasingly be challenged to meet the world’s needs for food and enhance and maintain the integrity of natural systems. Using plants, such as endophyte-infected tall fescue, that thrive under stressful extremes including drought and heavy grazing is one way producers can meet both environmental and economic objectives. Findings from my studies are important for land managers who must find alternative methods of eliminating invasive plant species, that more than likely contain high amounts of SC.

Plants often contain high amounts of SC, which limit intake by herbivores in monotonous diets, but results of my research suggest ways to encourage intake of SC by herbivores through planting complimentary mixtures of plants. Discovering which plants complement one another is an opportunity for researchers and producers. My studies provide important findings on a topic little studied. More research is needed to discover other possible SC combinations, and to assess impacts of planting SC mixtures on pastures. These findings will hopefully stimulate further research on possible benefits of SC and their influence on animal grazing behavior.

There is growing interest in reconstructing ecosystems to enhance ecological, economic, and social values. Yet, to do so, we must find ways to enhance biodiversity, environmental quality and the sustainability of grazing lands. In all these instances, plants are the glue that binds soils, water, herbivores, and people. However, monocultures or simple grass-legume mixtures are not always ideal for intensively managed pastures due to their seasonality, susceptibility to pests, and monotony of primary (nutrient) and secondary compound profiles. Diverse mixtures of plants may provide many benefits monocultures cannot.

As animals eat a wide variety of foods, some of which are complimentary, they are able to tolerate higher levels of SC, and make better use of PC, consequently spreading the load of herbivory across a landscape more evenly. The ability of animals to utilize complimentary foods enhances the biodiversity of plants and productivity of herbivores while decreasing a reliance on herbicides and insecticides. Complimentary compounds allow animals to ingest foods that otherwise would be avoided, and thus make use of weedy plant species that often overtake sensitive landscapes. Diverse arrays of SC also have implications for enhancing animal health through the use of foods not only as a physiological need but for medicinal purposes as well. More research is needed to investigate the medicinal use of SC in foods by animals, and the implications this knowledge has for managing livestock and wildlife systems.

Animal grazing behavior can be used as a valuable tool in managing our natural resources and landscapes, one that does not rely heavily on chemical enhancements and fossil fuels. More research in the area of SC complimentarily, as well as PC and SC interactions, is crucial to enhancing the health and vitality of plant, animal, and human systems. A better understanding of how these compounds interact within a complex network of ecological and behavioral systems is vital to a better future.

Results of my research suggest ways to encourage intake of SC by herbivores through planting complimentary mixtures of plants, and discovering which plants complement one another is an opportunity for researchers and producers. More research is needed to discover other possible SC combinations, and to assess impacts of planting SC mixtures on pastures. These findings will hopefully stimulate further research on possible benefits of SC and their influence on animal grazing behavior.

While human societies have dealt with nature's cornucopia in many ways, over time we have targeted only a handful of food species -- those that were abundant, palatable, easily cultivated and harvested -- for use in animal and human diets (Etkin, 1994). By focusing on a few species, people transformed the diverse world of plants into a manageable domain that generally met needs for energy and limited intake of toxins (Johns, 1994). In attempting to provide food for burgeoning populations, we have selected for a biochemical balance in crops and forages favoring primary compounds and nearly eliminating SC, which we have come to view generally as toxins with bitter flavors (Drewnowski and Gomez Carneros, 2000). To increase intake of single-plant diets, one must reduce SC as they limit how much of any one food humans and livestock can consume (Provenza et al., 2003). The outcome is energy- and protein-rich monocultures of plants low in the SC. This reduces the so called “problems” associated with secondary compounds, but creates a bigger one of reliance on fossil fuels, chemical insecticides, herbicides and fertilizers, pharmaceuticals, and overall declining health. The alternative, which we have not pursued, is offering animals and people a variety of forages that differ in both primary and secondary compounds, thereby enabling them and us to obtain a much greater array of nutrition, health and environmental benefits from nature’s pharmaceutical bounty. To create and sustain bio-diverse landscapes and food systems, we should be asking how and why nature grew plants in diverse mixtures and re-constructing grazing lands and dinner tables with assorted species of plants that provide complementary benefits for soils, plants, herbivores and people.

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